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Action Potentials Recorded Intra- and Extracellularly from the Isolated Frog Muscle Fibre in Ringer's Solution and in Air.

By

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Received 20 February 1957.

Volume conduction determines the shape of the external action potential from a potential source surrounded by a conducting medium and its change in amplitude and duration with varying distance from the source. Volume conduction has been analysed by LORENTE DE NÓ (1947 b) in the case of whole nerve but a corresponding analysis is lacking for muscle.

In the present study the action potential inside and outside the isolated muscle fibre in Ringer's solution was measured and the resultant potential field around the fibre was determined. For comparison the action potential was recorded from the surface of a fibre surrounded by a moist gas mixture (air).

Method.

Fibres from the frog's m. semitendinosus (*Rana temporaria*) were isolated and transferred to an experimental chamber containing Ringer's solution. The fibres, about ten mm in length, were stimulated at the one end and the action potential was recorded by intracellular or extracellular electrodes placed distal to the point of stimulation. The technique of preparation, the method for stimulation and for extracellular recording and the composition of the Ringer's solution have been described (HÅKANSSON 1956).

Intracellular recording. For intracellular recording a glass capillary microelectrode was used with an external tip diameter of less than $0.5\ \mu$ (LING and GERARD 1949). The electrodes were filled with a 3 M KCl solution (NASTUK and HODGKIN 1950) and their impedance was

¹ Working under a grant from the Rask-Ørsted Foundation.

about ten Megohms. The rise time of a square wave pulse through the electrode and amplifier ("time constant") was 70 μ sec. This rather long rise time was due to the fact that the tip of the microelectrode dipped at least 1.5 mm in the Ringer's solution covering the muscle fibre.

Recording from fibres suspended in a moist chamber. To obtain action potentials from an insulated fibre, recordings were made from the fibre surrounded by a moist gas mixture consisting of 99 per cent oxygen and one per cent carbon dioxide. Fibres in the horizontal position adhered to the fluid surface and were damaged when the Ringer's solution was replaced by the gas mixture. The damage could be avoided if the fibre was suspended vertically. The film of Ringer's solution which adhered to the fibre after emptying the chamber was so thin that there resulted a high external resistance (p. 308). In order to prevent the fibre from drying it was necessary to maintain a humidity of 100 per cent in the chamber. To this purpose the gas mixture was bubbled through four successive bottles of hot Ringer's solution. Just before entering the experimental chamber the warm moist gas mixture was cooled to the temperature of the chamber. To test that the humidity was no less than 100 per cent a piece of wet cotton wool was placed on a thermistor inside the chamber. If this thermistor registered a lower temperature in moist air than a thermistor placed close to the muscle fibre, evaporation had occurred from the cotton wool and the humidity was less than 100 per cent. To avoid accumulation of potassium (p. 309) in the Ringer's film surrounding the muscle fibre, Ringer's solution was washed through the chamber about every 15 minutes.

Oxygen has been shown to act as a poison to nerve-muscle preparations (BEAN and BOHR 1938) and to nerve (PEROT and STEIN 1956). The possibility that the high oxygen concentration in the gas surrounding the muscle fibres in the present experiments might interfere with the function of the fibre was investigated in ten experiments in which the gas mixture consisted of 20 per cent oxygen, 79 per cent nitrogen and one per cent carbon dioxide. The survival time of the fibres was identical in the two gas mixtures indicating that the high oxygen concentration did not act as a cell poison in the experiments reported.

Survival time of the fibres in the gas mixture. With a stimulation frequency of less than one in five seconds the conduction velocity and amplitude could remain constant for as long as three hours, longer than when it was surrounded by a large volume of Ringer's solution. With a stimulation frequency of more than two per second there was a rapid slowing in conduction velocity and a decrease in action potential amplitude. Restitution occurred with the cessation of stimulation and was accelerated by washing the fibre in Ringer's solution.

The recording electrodes consisted of thin silver plates each with a hole of 1.5–2 mm in diameter through which the fibre passed (Fig. 1). Contact between the fibre and the silver plate was by means of a thin Ringer's film in the hole maintained by surface tension. The contact area on the fibre was seen to be less than 100 μ in the longitudinal direction. The advantages of this type of electrode were the low impedance, not exceeding 1×10^5 ohms; the small contact area on the fibre and the

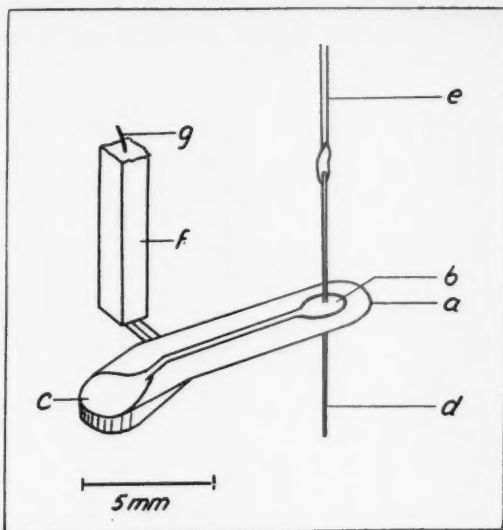


Fig. 1. Electrode for the recording of action potentials from a muscle fibre suspended in air.

- a) Silver plate, 50 μ thick.
- b) Hole through which the fibre passes. The hole is covered by a thin film of Ringer's solution which makes contact around the fibre.
- c) Droplet of Ringer's fluid to maintain the film.
- d) Muscle fibre.
- e) Glass rod to which the muscle fibre is attached.
- f) Perspex pin.
- g) Lead to amplifier.

absence of a damaging contact. Four electrodes were used, each attached to its own perspex pin (f in Fig. 1) which could be moved parallel to the longitudinal axis of the fibre. During experiments in Ringer's solution the electrodes were pulled up around the glass rod. For recording in the gas mixture the chamber was placed vertically and the fluid level lowered. This operation caused a thin film of Ringer's fluid to remain in the hole in the silver plates. When the muscle fibre was entirely surrounded by the gas mixture, the electrodes were pushed down around the fibre to a suitable level. The two electrodes situated close to the stimulated end of the fibre were coupled to one differential amplifier, the two other electrodes to a second differential amplifier. Usually the two electrodes in a pair were separated by one mm, the two pairs by 3—4 mm to allow measurement of conduction time.

To reduce the *stimulus artifact* during experiments in the gas mixture the bridge arrangement was used shown in Fig. 2 whereby the voltage (V) from the stimulus common to both input terminals of the differential amplifier could be balanced to a minimum value.

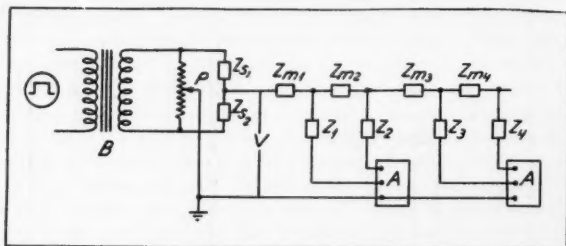


Fig. 2. To reduce the stimulus artifact when recording in air.

A. Differential amplifier.

B. Stimulation transformer.

The voltage (V) from the stimulation circuit common to both input terminals of the differential amplifier can be balanced to minimum by the potentiometer (P).

The rectangles indicate the impedances of the stimulating electrodes (Z_{s1} , Z_{s2}), the recording electrodes (Z_1 — Z_4) and the muscle fibre (Z_{m1} — Z_{m4}).

Results.

Extracellular Action Potential Recorded in a Large Volume of Ringer's Solution at Various Distances from the Fibre.

The external action potential was recorded at various distances between $30\ \mu$ and $1,500\ \mu$ from the fibre in 50 — $100\ \mu$ steps at right angles to it. The external electrode was advanced no closer than $30\ \mu$ from the surface of the fibre because of the danger of damage when the contracting fibre moved. At distances of more than $1,500\ \mu$ the action potential was too small to be discriminated against the noise level. An example of the recorded potentials at 30 , 150 and $460\ \mu$ from the fibre surface is shown in Fig. 3. The amplitude of the negative deflection was slightly higher than that of the positive. The decrement in amplitude of the positive and negative phases with increasing distances is shown in Fig. 4. Near the surface of the fibre the amplitude decreased rectilinearly with the logarithm of the distance from the fibre axis (Fig. 4 inset). At about $600\ \mu$ from the fibre the amplitude was only one tenth its value at $30\ \mu$. When both amplitude and distance were plotted on a logarithmic scale the amplitude decreased nearly rectilinearly with distance for distances larger than $0.15\ \text{mm}$ from the axis of the fibre. The relationships for fibres of different circumferences were essentially similar, the amplitude being the higher the larger the circumference (Fig. 5). The parallel course of the different amplitude-distance relationships indicates that the relative

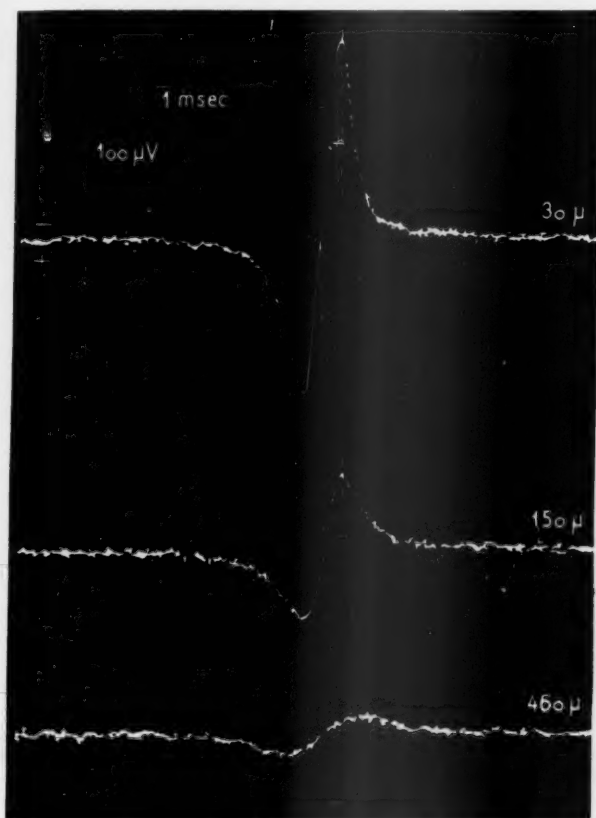


Fig. 3. Action potential from an isolated muscle fibre, 352 μ in circumference, in Ringer's solution, recorded at 30 μ , 150 μ and 460 μ from the surface of the fibre (22° C). The dotted lines connect the positive and negative peaks and indicate the increase in duration of the positive-negative deflection with increasing distance.

decrease in amplitude is identical for fibres of different circumference. At the same distance from the axis of the fibre the amplitude for the different fibres varied as the square of the radius.

The time interval between the positive and negative peaks of the action potential increased with increasing distance from the muscle fibre: A duration of the steep positive-negative deflection of for

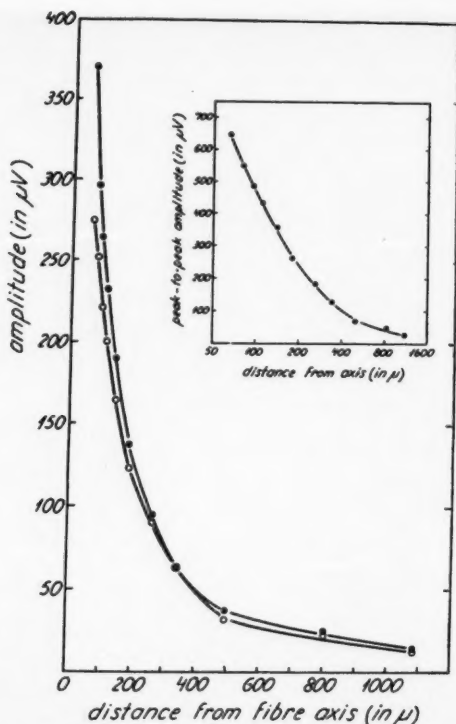


Fig. 4. Amplitude of first, positive ($\circ-\circ-\circ$) and second, negative ($\bullet-\bullet-\bullet$) phase of the external action potential from an isolated muscle fibre in Ringer's solution, plotted against the distance between recording electrode and fibre axis. Inset: plot of peak to peak amplitude against the logarithm of the distance from the axis of the fibre.

example 270 μsec (Fig. 3, 22° C) was found with the electrode 30 μ from the surface of the fibre, at a distance of 500 μ twice this duration was recorded.

Spread of Equipotential Lines in a Conducting Medium Surrounding the Fibre.

From the active part of a muscle fibre in Ringer's solution the current spreads outwards in the surrounding medium. Equipotential lines were constructed from the action potentials measured at different distances from the fibre (Fig. 6). The current passes perpendicularly to these equipotential lines. The equipotential

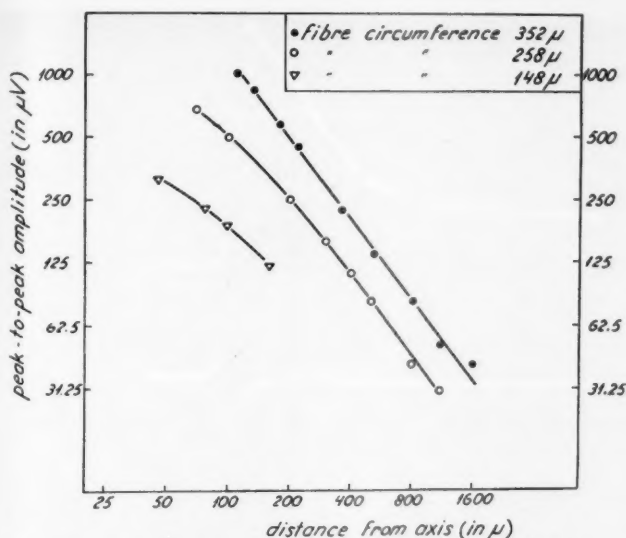


Fig. 5. Action potential amplitude as a function of the distance from the axis of the fibre immersed in Ringer's solution for fibres of different circumference (logarithmic scales, 20–22° C).

line for zero potential was perpendicular to the axis of the muscle fibre. The equipotential lines for the positive and negative phase of the potential spread nearly symmetrically around the zero line with a slightly larger spread of the positive phase. For potentials exceeding 100 μ V the spread along the fibres was 0.7 mm to both sides from the point of zero potential; in the transverse direction it was 0.3 mm from the surface.

From the travelling field (Fig. 6) the total current was estimated which passes the membrane of the muscle fibre during activity. To this purpose the radial component of the field strength (E_r) was determined at a distance of 100 μ from the surface of the fibre. The radial current density (I_r) is then

$$I_r = \sigma \cdot E_r$$

where σ is the conductivity of the Ringer's solution surrounding the fibre ($\frac{1}{\sigma} = 83$ ohm cm, 20° C; KATZ 1948). The maximum current density 100 μ from the surface was about 0.3 milliampere per cm². The currents (i) passing through different cylindrical segments are

$$i = I_r \cdot \Delta A$$

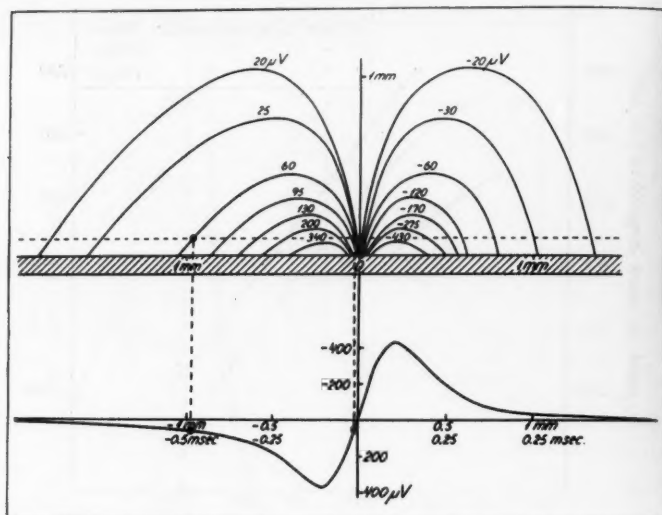


Fig. 6. Equipotential lines around the active muscle fibre in Ringer's solution. Two points (●) on an equipotential line (e. g. $+60 \mu\text{V}$) are obtained by projecting the two corresponding points of the action potential (below) on the line which corresponds to the distance at which the potential was recorded (broken line). The wave of activity can be regarded as moving along the fibre (hatched area) from right to left.

where ΔA is the surface of a given segment. The sum Σi of the currents over the positive or the negative part of the field represents the total current passing through the membrane. It amounted to 9×10^{-8} amp. With a conduction velocity of the action potential of 2.0 m per sec (20°C) the propagation along the fibre lasted 5 msec per cm fibre length and therefore the total electric charge passing across the membrane of the muscle fibre during activity was 4.5×10^{-8} coulomb per cm fibre length ($(9 \times 10^{-8}) \times (5 \times 10^{-3})$). Since a one cm fibre with a diameter of 100μ has a surface of $\pi \times 10^{-3} \text{ cm}^2$ the total charge passing the membrane in one direction during activity was 1.4×10^{-6} coulomb per cm^2 . This corresponds to the transport of 1.4×10^{-11} moles univalent ions per cm^2 .

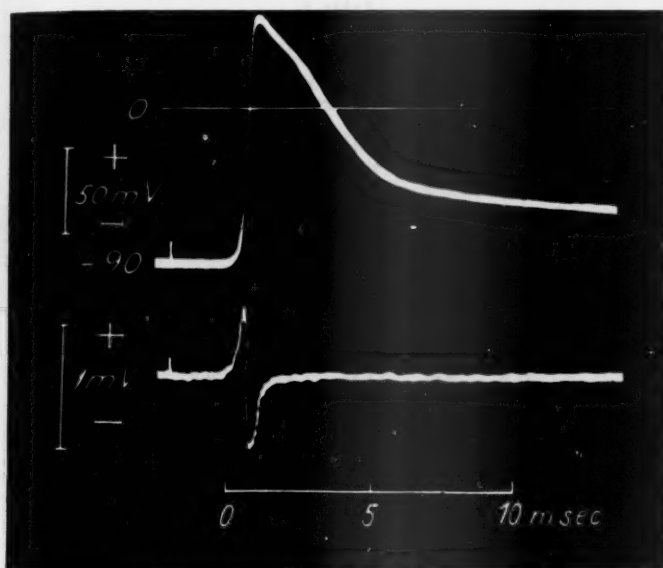


Fig. 7. Action potential of an isolated frog muscle fibre, surrounded by a large volume of Ringer's solution, recorded simultaneously with an intracellular electrode (upper beam) and with an extracellular electrode (lower beam). 19°C. Membrane potential 90 mV. S = stimulus.

Intracellularly and Extracellularly Recorded Action Potential of the Isolated Muscle Fibre in Ringer's Solution.

The intracellular action potential was recorded with an electrode inserted into the fibre just opposite the opening of an extracellular electrode placed as close as possible to the surface of the fibre. An example of a simultaneous record of these two action potentials is shown in Fig. 7. The monophasic, intracellular action potential had an amplitude varying in different fibres from 73 to 137 mV (Table 1). The mean value was 108 mV; corrected for the "time constant" (p. 292) of the recording device it was 112 mV. The time for the rise of the potential from 20 to 100 per cent of crest was 0.52 ± 0.04 msec (21.2° C), *i. e.* of a similar order of magnitude as that found by NASTUK and HODGKIN (1950, 0.49 ± 0.01 msec at 17.4° C); and NASTUK (1953, 0.33 ± 0.006 msec, 22.6° C). The total duration of the intracellular action potential could not be determined with certainty on account of the protracted course

Table 1.

Comparison between the monophasic spike of a muscle fibre in a gas mixture (above) and the intracellular spike recorded with the fibre in Ringer's solution (below).

External medium	Temperature	Circumference	Conduction velocity	Duration from 50 per cent rise to 50 per cent fall	Duration ² × conduction velocity	Time for rise from 20–100 per cent of crest	Amplitude
	°C	μ	m/sec	msec	mm	msec	mV
Gas mixture	19.8	235	0.9	2.8	2.5	0.32	95
	20.5	256	0.8	3.2	2.6	0.35	109
	21.0	275	1.2	3.1	3.7	0.20	122
	20.0	330	0.9	3.7	3.3	0.33	84
	21.0	336	1.25	1.75	2.2	0.19	111
	21.0	368	1.08	3.3	3.6	0.29	115
	22.0	383	1.2	3.0	3.6	0.21	109
	20.1	—	—	2.3	—	0.30	118
Mean value	20.7	312	1.05 ±0.07	2.9 ±0.2	3.1 ±0.2	0.27 ±0.02	108 ±4
Ringer's solution	21.2	300	1.90 ¹	2.9	5.5	0.50	73
	21.0	315	1.95	3.0	5.9	0.60	94
	20.9	354	2.30	3.3	7.6	0.45	94
	21.5	384	2.50	4.5	11.3	0.67	137
	21.0	404	2.55	—	—	0.45	106
	20.6	412	2.55	3.2	8.2	—	108
	22.0	415	2.75	4.3	11.8	0.47	128
	21.9	428	2.80	4.0	11.2	—	120
Mean value	21.2	377	2.41 ±0.12	3.6 ±0.25	8.8 ±0.9	0.52 ±0.04	108 ±7

¹ Calculated from HÅKANSSON (1956), Fig. 6, II A.

² Duration from 50 per cent rise to 50 per cent fall.

of the repolarisation phase. The time interval between the rise from and fall to 50 per cent of crest was 2.9 to 4.5 msec with a mean value of 3.6 ± 0.25 msec. With a conduction velocity of 2.4 m per sec (21° C) this part of the potential is spread over a length of 8.6 mm along the fibre.

About two msec after the peak of the action potential the slope increased rather suddenly (Fig. 7). This change in slope is not identical with the more pronounced hump found by FATT and KATZ (1951) and NASTUK (1953) due to a superimposed end-plate potential. The present experiments were performed on directly stimulated and fully curarized fibres with the recording electrode 7–8 mm from the stimulating electrode. Since the

experiments were made on isolated fibres distortion from adjacent fibres (EASTON 1954, 1955, 1956) can be excluded as well.

The external action potential recorded in Ringer's solution was diphasic, the first phase positive and the peak to peak amplitude 0.8–2.9 mV, mean value 1.8 mV (7 potentials). The intra- and extracellularly recorded potentials had a simultaneous onset and the negative peak of the externally recorded potential was simultaneous with the peak of the intracellular potential. The duration of the diphasic response was 2.67 ± 0.14 msec; *i. e.* less than 1 msec longer than the rising phase of the intracellular action potential. The duration of the diphasic potential increased slightly with increasing fibre circumference, from 2.5 msec at 150 μ circumference to 3.2 msec at 350 μ circumference (21° C).

The time relationship between the extracellular action potential and the intracellularly recorded potential indicates that the extracellular action potential can be accounted for in terms of the second derivative of the intracellular action potential (cf. LORENTE DE NÓ, 1947 b): The positive peak of the extracellular potential occurs at the time the rising phase of the intracellular potential displays the most pronounced change in slope, just as the negative peak of the extracellular potential coincides with the peak of the intracellular potential. Since the course of repolarisation is very gradual, much more so than in nerve, it does not manifest itself by a third positive phase in the extracellular potential.

Action Potentials from a Muscle Fibre Surrounded by Air.

The monophasic action potential could not be measured directly from the muscle fibre suspended in a moist gas mixture: the potential led off by the first electrode had not yet disappeared when the impulse reached the second electrode, even at the tendon end of the muscle fibre. The diphasic potential recorded by a pair of electrodes represents the difference between the monophasic potentials picked up by each electrode of the pair (Fig. 8). These potentials are displaced by an interval corresponding to the time required for the action potential to propagate from one electrode of the pair to the other. Recordings were made with interelectrode distances of one to four mm. With a propagation velocity of about 1 m per sec this corresponds to a displacement (Δt) of 1 to 4 msec. The duration of the ascending phase of the action potential was the same independent of the electrode distance and therefore

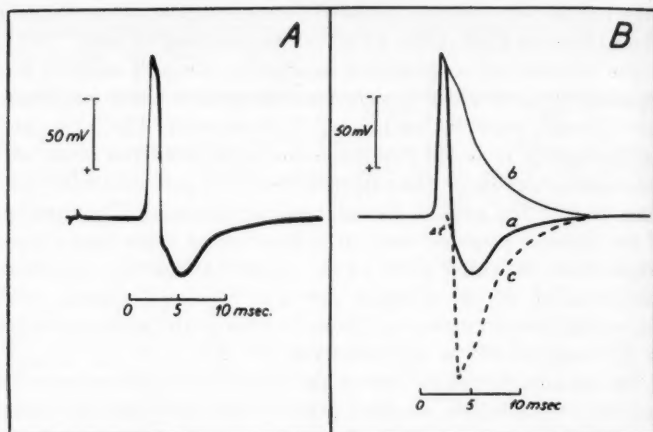


Fig. 8. A. Action potential recorded from a muscle fibre in air. The distance between the recording electrodes was 1.2 mm; the conduction velocity 0.8 m per sec (20.5°C). B. Graphically constructed monophasic response. a) diphasic surface action potential, b) reconstructed monophasic potential, c) monophasic potential recorded by the second electrode of the pair (reversed).

this phase and part of its decline in the diphasic potential were identical with these portions in the extracellular monophasic potential. However, the reconstruction of the monophasic potential is complicated by the fact that the descending phase reconstructed from recordings with short distances between the electrodes of the pair is different from that which is recorded with long distances (Figs. 8 and 9).

The amplitude of the initial phase of the extracellular diphasic potential was 108 ± 4 mV, *i. e.* of the same order of magnitude as that of the intracellularly recorded action potential (Table 1). This high amplitude was found even with a distance between the leading-off electrodes of as little as 1 mm and it indicates that under these conditions the rising phase of the potential is spread over a range of less than one mm along the muscle fibre. The rise time from 20 to 100 per cent of the crest was 0.27 ± 0.02 msec (8 fibres, 20.7°C). This was shorter than the rise time of the intracellular action potential with the fibre in Ringer's solution (0.52 ± 0.04 msec, 21.2°C , Table 1). The longer rise time found in intracellular measurements cannot be accounted for by the distortion due to the time constant of the microelectrode and amplifier (p. 292). Correcting for the distortion (SOLMS, NASTUK

and ALEXANDER 1953) the actual intracellular rise time was about ten per cent shorter than the measured value and the peak amplitude was on the average measured three per cent too low. The total duration of the diphasic response is no suitable expression of the duration of membrane changes since the protracted terminal course of these changes is largely cancelled by the bipolar recording.

The later portions of the monophasic surface action potential of a fibre in air can be constructed graphically from the diphasic potential if the conduction velocity is known (LORENTE DE NÓ 1947 a). The conduction velocity was determined from the arrival time of the spike potential at the two pairs of electrodes and from the distance between the pairs. Thereby the displacement Δt due to propagation from the first to the second electrode of a pair could be determined with greater accuracy than from the diphasic potential itself. The graphically reconstructed monophasic potential is shown in Fig. 8 B. The monophasic potential and the first portion of the diphasic potential being identical over Δt , the course of the monophasic potential up to $2 \Delta t$ is obtained by adding the initial portion of the diphasic potential to the diphasic potential after a displacement in time Δt . Then, by addition of the monophasic potential known up to $2 \Delta t$, we obtain its course up to $3 \Delta t$ and so on.

The total duration of the reconstructed monophasic surface potential cannot be evaluated with sufficient accuracy since the terminal part of the potential is lost in a bipolar recording. With a distance between the leading-off electrodes of about 1 mm the duration measured from 50 per cent of rise to 50 per cent of fall averaged 2.9 ± 0.2 msec (20.7°C); there was a tendency for the reconstructed monophasic potential in an insulating medium to have a shorter duration than the intracellularly measured potential with the fibres in Ringer's solution (Table 1).

It is surprising that the first phase of the diphasic potential recorded with a large electrode distance differed considerably from that to be expected from the monophasic potential reconstructed from a recording with a short distance between the electrodes: 4 msec after the start of the initial deflection the potential recorded with a large distance between the electrodes had decreased by 25 per cent (Fig. 9) while the monophasic potential reconstructed from recordings with a short distance between the electrodes had fallen 55 per cent (Fig. 8 B). In addition, with large distances

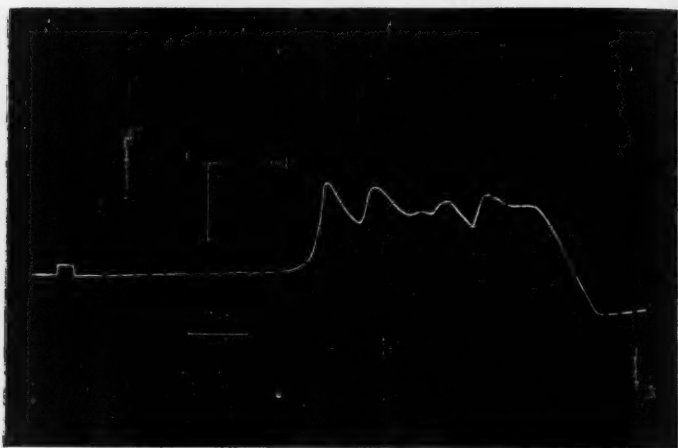


Fig. 9. Initial phase of an action potential from a muscle fibre in air, recorded by two electrodes 4 mm apart. The conduction velocity was 1.08 m per sec (21° C).

between the electrodes (4 mm) irregularities were seen in the diphasic potentials which did not appear with short distances (1—2 mm). They are illustrated in Fig. 9 which shows the first phase of a diphasic response. The base line with the fibre at rest had no trace of oscillations. Therefore, they cannot be caused by potential changes on the second electrode to which the action potential had not yet arrived. Since the action potential represents the potential changes which occur at the site of the electrode the irregularities cannot either be due to unevenness in the fluid film along the fibre. Hence, the irregular oscillations represent potential changes occurring in the muscle fibre membrane at the site of the first electrode of the pair and indicate repeated partial re- and depolarizations of the membrane. We have no explanation to offer for the longer duration of the monophasic potential obtained with long interelectrode distance as compared with that found with short interelectrode distance and for the irregularities in the repolarization phase.

The *conduction* of the action potential over the muscle fibre proceeded at about half the velocity when the fibre was placed in a moist gas chamber than when it was surrounded by a large volume of Ringer's solution. The velocity was 1.05 m per sec

(s. d. 0.18 m per sec, 20.7° C) with the fibre in air while it was 2.4 m per sec (s. d. 0.34 m per sec, 21.2° C) in Ringer's solution (Table 1). Correspondingly, it is seen that the conduction velocity for fibres of different circumferences in air varied by about half as much as in Ringer's solution. On account of this low conduction velocity the action potential with the fibre in air is spread over a shorter distance than it is in Ringer's solution. Constructed from measurements with a 1 mm distance between the two electrodes of the pair the monophasic action potential in air spread over 3.1 mm (50 per cent rise to 50 per cent fall) while the spread was 8.8 mm in Ringer's solution.

Discussion.

The membrane changes occurring during impulse propagation in an excitable tissue manifest themselves in the action potentials recorded intracellularly and extracellularly. The amplitude of the volume conducted action potential from a frog muscle fibre in a large volume of Ringer's solution decreased markedly with increasing distance from the fibre, the decrease being rectilinear with the logarithm of the distance near its surface while at larger distances (d) from the axis of the fibre the amplitude decreased as $d^{-1.5}$. The spread of action currents outside an excitable cell in situ or in an artificial medium depends on the potential source as well as on the external medium. For muscle fibres of different diameter the relative decrease in amplitude as a function of distance was the same, the absolute amplitudes varying as the square of fibre diameter.

The effect of both distance and size of the potential source on the amplitude of the volume conducted muscle fibre action potential is in agreement with LORENTE DE NÓ's (1947 b) calculations for the action potential of nerve. He calculated the volume conducted action potential from the potential recorded on the surface of the nerve when it was surrounded by an insulating medium. The potential V in a point P of the conducting medium was:

$$V(P) = - \frac{A}{4\pi} \int \frac{d^2 V_e}{dz^2} \cdot \frac{1}{r} dz$$

where A is the cross sectional area of the nerve, V_e the surface action potential with the nerve in an insulating medium and r the distance between P and the points on the axis of the nerve (z).

A linear decrease in amplitude with the logarithm of distance from the axis can be deduced from this expression if the distance is small as compared with the depolarized section along the nerve. It can furthermore be seen that the amplitude is proportional to the cross sectional area (A) of the source if the surface action potential is the same for fibres of different diameter.

There is an essential difference in the volume conducted action potential of nerve and muscle fibres: the former being triphasic and the latter diphasic. In nerve the third positive phase is substantially lower than the two preceding phases of the potential. That it is absent in the action potential of the muscle fibre indicates a more gradual course of repolarization. This is actually found in intracellular measurements: the ratio between the duration of the rising and the descending phase of the monophasic action potential being 10 in the muscle fibre and 1.4 in the squid giant axon (HODGKIN and KATZ 1949) when measured between 50 per cent of rise and 50 per cent of fall.

In analogy to measurements on a whole nerve (LORENTE DE NÓ 1947 b), it was originally intended to use the action potential measured with the muscle fibre in air to deduce properties of the volume conducted potentials in Ringer's solution. However, a prerequisite for this procedure would be that the potential sources remain unaltered when moved from the insulating medium into a volume conductor. In fact, this does not apply to the action potential sources of the muscle fibre: the spread of the monophasic potential along the fibre is considerably shorter with the fibre in air than in Ringer's solution. The change is due to the high impedance of the very thin Ringer's film surrounding the muscle fibre and one might expect better agreement between the spread in air and in Ringer's solution were the Ringer's film thicker. On the other hand, the time relationship of the action potentials measured extracellularly and intracellularly with the fibre in Ringer's solution suggests that the extracellular potential is related to the second derivative of the intracellular potential in Ringer's solution. This agrees with the general concept of the membrane theory in that the membrane currents are proportional to the second derivative of the intracellular action potential (cf. HODGKIN 1954).

It would have been desirable to compare the results of volume conduction of the muscle fibre action potential presented here with those from isolated non-myelinated nerve fibres. However,

experiments of this type are not available. Therefore, a comparison has to be confined to their surface action potentials in an insulating medium. In a non-myelinated crab nerve fibre the spread of the action potential along the fibre was calculated to two mm with the fibre in an insulating medium (50 per cent rise to 50 per cent fall, HODGKIN 1939), *i. e.* of the same order of magnitude as in the isolated muscle fibre in air. In the squid giant axon of 500 μ diameter the spread was 10 mm (HODGKIN 1939). LORENTE DE NÓ (1947 a, b), though working with a whole myelinated nerve, used a strength of stimulation to activate a homogeneous group of alpha fibres; therefore, the spread of the action potential along the nerve obtained in his experiments may be considered very close to that of an isolated nerve fibre. Determined from the surface action potential in an insulating medium it was 21 mm, *i. e.* about seven times that of the single muscle fibre in moist air and more than twice that of the muscle fibre in Ringer's solution.

A simultaneous recording of the extracellular volume conducted action potential and the intracellular potential showed that the total duration of the extracellular potential is only slightly longer than that of the rising phase of the intracellular potential. The major part of the repolarization phase is thus not reflected in the external volume conducted potential.

The surface action potential in an insulating medium was propagated with much lower velocity than that in Ringer's solution. This reduction in velocity is due to the high external resistance (HODGKIN 1939). A direct measurement of the external resistance with the fibre in air was complicated by the shunt effects arising from the interior of the muscle fibre. From the conduction velocities in Ringer's solution and in air it may be estimated. With a specific resistance of the fibre interior of 230 ohm cm (KATZ 1948) the longitudinal resistance per cm (r_i) for a fibre of 100 μ diameter is 2.9×10^6 ohm per cm. The relationship between propagation velocity (V) and the external (r_e) and internal longitudinal resistance (r_i) per cm is given by

$$V = \frac{K}{\sqrt{r_e + r_i}} \quad (\text{RUSHTON 1937})$$

where K is a proportionality factor. With a propagation velocity in Ringer's solution of 2.4 m per sec and in air of 1.05 m per sec the external resistances are related by

$$\frac{1.05}{2.40} = \frac{\sqrt{r_i + r_e(\text{Ringer})}}{\sqrt{r_i + r_e(\text{air})}}$$

From this the external resistance r_e in air is calculated

$$r_e(\text{air}) = 11.5 \times 10^6 \text{ ohm per cm} + 5 r_e(\text{Ringer})$$

The specific resistance of Ringer's solution is 83 ohm cm (KATZ 1948). The current flow being concentrated within about 300 μ from the axis of the fibre (Fig. 4) the external longitudinal resistance with the fibre in Ringer's solution is roughly:

$$r_e(\text{Ringer}) = \frac{83}{\frac{\pi (600^2 - 100^2) \cdot 10^{-8}}{4}} \text{ ohm per cm}$$

i. e.

$$r_e(\text{Ringer}) = 3 \times 10^4 \text{ ohm per cm.}$$

With this external resistance in Ringer thus a value in air of about 12×10^6 ohm per cm is obtained. The thickness of the Ringer's film (Δd) surrounding the muscle fibre in the moist gas mixture was estimated as follows:

$$r_e(\text{air}) = \frac{r_{\text{spec}}(\text{Ringer})}{a}$$

where a is the cross sectional area of the film of Ringer's solution.

$$a = \Delta d \cdot \pi \cdot d$$

where d is the diameter of the muscle fibre. Substituting the value for the external resistance calculated on the basis of the propagation velocity, the thickness of the film of the Ringer's solution would be 2.3 μ .

From the potential field around the fibre, the conductivity of the surrounding medium and the conduction velocity of the action potential, the total charge was determined which passes the membrane of the muscle during activity. It amounted to 1.4×10^{-6} coulomb per cm². The total charge which passes the partially curarized motor end-plate during the end-plate potential is 8×10^{-10} coulomb (FATT and KATZ 1951). The end-plate potential is detected over about two mm along the muscle fibre (FATT and KATZ 1951), corresponding to a surface area of 6×10^{-3} cm².

Hence, the ionic flux during the end-plate potential is 1.3×10^{-7} coulomb per cm^2 . This is about ten times smaller than the flux estimated to pass the muscle fibre membrane during activity. Expressed in moles of univalent ions per cm^2 the flux through the muscle membrane amounted to 1.4×10^{-11} . In single nerve fibres the flux was about three to five times smaller (KEYNES and LEWIS 1950).

As to the interpretation of the potential changes in terms of membrane processes, the rising phase of the intracellular action potential has been correlated to an increase of the membrane permeability to sodium ions (HODGKIN, HUXLEY and KATZ 1950). An active membrane process has been supposed to reduce sodium entry and to rebuild the membrane potential. The change in the permeability of the membrane to sodium and potassium ions is related but displaced in time. The exit of potassium ions from inside to outside tends to reduce the action potential and might possibly cause a dip in the repolarization phase (cf. HODGKIN 1950, KEYNES 1950). In analogy to these interpretations the several dips and humps recorded in the repolarization phase with the fibre in air might indicate repeated changes in permeability. Changes in excitability of oscillating nature has been observed in nerve (ARVANITAKI 1939, HODGKIN, HUXLEY and KATZ 1950, MONNIER 1952) and could be influenced by the potassium and calcium content of the surrounding medium. Possibly in the case of the muscle fibre output and uptake of potassium by each stimulus tends to give a re- and depolarization of the membrane. The regenerative tendency might be magnified in the air experiments since the thin Ringer film outside the fibre allows the change in ion concentration to be greater than in the experiments in Ringer's solution.

Summary.

Action potentials were recorded inside and outside isolated frog muscle fibres in Ringer's solution and in moist air in order to correlate the different potentials and to study volume conduction.

The *extracellular action potential* recorded from isolated frog muscle fibres in Ringer's solution was diphasic with a first positive and a second negative phase.

The duration of the potential measured at a distance of 30μ from the surface of the fibre was 2.67 ± 0.14 msec (21.2°C).

It increased slightly with increasing fibre circumference from 2.5 msec in a fibre of 150 μ circumference to 3.2 msec in a fibre of 350 μ circumference.

The amplitude increased proportionally to the square of the fibre circumference. The peak to peak amplitude was 0.8 to 2.9 mV 30 μ from the surface of the fibre. Near the fibre the amplitude decreased linearly with the logarithm of the distance from the axis of the fibre. At distances (d) exceeding 0.15 mm from the axis of the fibre the amplitude decreased as $d^{-1.3}$.

The peak of the *intracellularly* measured potential coincided with the negative peak of the extracellular potential with the fibre in Ringer's solution. This indicates that the extracellular action potential can be accounted for by means of the second derivative of the intracellular potential. A third positive phase of the volume conducted potential which is present in nerve disappears within the noise level for the muscle fibre in Ringer's solution because of the more protracted course of repolarization.

The spread of the action potential along the fibre determined from the intracellularly recorded action potential was 8.8 ± 0.9 mm (conduction velocity \times duration of the potential from 50 per cent rise to 50 per cent fall).

From the action potentials recorded at different distances from the fibre equipotential lines of the travelling potential field were constructed. By means of this field the charge passing the membrane during depolarization was determined to 1.4×10^{-6} coulomb per cm^2 .

The surface action potential from a fibre surrounded by a moist gas mixture had an amplitude of the same order of magnitude as the intracellularly measured action potential with the fibre surrounded by Ringer's solution. The time for rise from 20 to 100 per cent of crest was about half the rise time of the intracellularly recorded potential. The duration of the reconstructed monophasic potential from 50 per cent rise to 50 per cent fall was slightly shorter than the intracellular action potential.

The conduction velocity of the potential with the fibre in air was half that measured in Ringer's solution (2.41 ± 0.12 m per sec in Ringer's solution; 1.05 ± 0.05 m per sec in air at 21°C). From this difference in propagation velocity the impedance of the Ringer's film surrounding the fibre in air was estimated to 12 megohms per cm. The thickness of the Ringer's layer was about two μ .

Grateful acknowledgement is made to Professor F. BUCHTHAL for his helpful criticism and support, to POUL ROSENFALCK, M. Sc. for his help in the theoretical elaboration of the problem and to O. STEN-KNUDSEN, M. D. and to CHR. GULD, E. E. for valuable discussions.

The work has been supported by the *Michaelsen Foundation* and a *Lilly Research Grant*.

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**Potentiation by Histaminase Inhibitors of the
Effects of Histamine on the Rectal Temperature
in the Unanaesthetized Guinea-Pig.**

By

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Received 26 February 1957.

In previous work it was observed that the effects of histamine on the respiratory movements of unanaesthetized guinea-pigs were potentiated by histaminase inhibitors (WESTLING 1956). It seemed to be of interest to find out whether also other effects of histamine on intact guinea-pigs were modified by inhibitors of histaminase. In the experiments to be presented the effect of histamine on the rectal temperature has been studied.

Histamine has been found to decrease the rectal temperature in various species (DALE and LAIDLAW 1911, PACKMAN, ROSSI and HARRISSON 1953). The mechanism for the fall in rectal temperature after histamine is not fully known. An increased heat loss has been observed, but diminished heat production is probably also responsible (*i. a.* COLLEDAHL 1943, BEZNÁK, KOVÁCH and GÁSPÁR-RÁDY 1948, GYERMEK 1950, ISSEKUTS, LICHTNECKERT and NAGY 1950 and FABINYI-SZEBEHELY and SZEBEHELY 1953).

Experimental.

Animals. Male guinea-pigs with an initial body weight of 325–425 g were obtained from a local dealer, keeping a closed colony. The animals were kept in air-conditioned quarters at 20° C.

The food was turnips, beets, hay and corn ad libitum. The animals were kept at the Institute for at least one week before being used in experiments.

Measurement of the rectal temperature. Each animal was put in a metal box, which was 15 cm long, 10 cm wide and 15 cm high. As a rule the animals were very quiet in these boxes. The experiments were performed in a room with a thermostatically controlled temperature of 20° C.

Thermocouples (applicator type RM 4, Elektrolaboratoriet, Copenhagen) with an external diameter of 2 mm were dipped in liquid paraffin and inserted 5 cm into the rectum. The applicators were kept in place throughout the experiment with strips of adhesive tape. The individual thermocouples were connected to a galvanometer (type TE 3, Elektrolaboratoriet) by means of a connexion box (type FE 10, Elektrolaboratoriet). The scale of the galvanometer was divided in 0.2° C, but readings could be taken to the nearest 0.1° C. The temperature of each animal was recorded every 10 minutes, starting 30 min. before any injections. A histaminase inhibitor or an equal volume of 0.9 % saline solution was injected 30 minutes before histamine or the substances with histamine-like actions. In a few experiments mepyramine was injected subcutaneously 30 minutes before histamine or one of the other substances.

The experiments were performed at the same time of the day, the thermocouples being inserted at about 9⁰⁰ a.m. Each animal was subjected to experiments every seven days. The experiments with potentiation of histamine effects by aminoguanidine were performed in July. The other experiments were made in September—November.

After a subcutaneous injection of histamine the rectal temperature started to fall after about 20 min. The maximal fall was usually seen after 30—90 minutes. Later on the rectal temperature rose to normal values. In some experiments the fall in rectal temperature induced by histamine was followed by a temporary rise to values above normal, but this feature was not taken in account. The data used were 1) the maximal fall in rectal temperature *i. e.* the difference between the temperature level before injection (usually 38.5°—39.0° C), and the lowest temperature recorded after histamine and 2) the duration of the response, which was taken to be the time interval between the injection of histamine and the first reading of restored normal temperature. In a few experiments, where the fall in rectal temperature was large, the temperature did not return to the normal level but was stabilized at a level somewhat lower than before injection. In these experiments the duration was measured from the injection to the first value at this lowered level.

Some guinea-pigs showed large spontaneous fluctuations in the rectal temperature in the first one or occasionally two experiments. Such fluctuations made the experiment impossible.

The measurements of the responses of the rectal temperature to 2- β -aminoethyl pyridine and 3- β -aminoethyl -1, 2, 4-triazole were made as described above for histamine.

*Substances and injections.** The following substances were used: histamine acid phosphate (British Drug Houses), 2- β -aminoethyl pyridine dihydrochloride, 3- β -aminoethyl-1, 2, 4-triazole dihydrochloride, aminoguanidine sulphate (Eastman Kodak), 2-methyl-4-amino-5-methylamino-pyrimidine dihydrochloride (called B₁pyrimidine) and mepyramine maleate (Merck & Co.). Dosages for histamine, 2- β -aminoethyl pyridine and 3- β -aminoethyl-1, 2, 4-triazole are given in terms of the bases, those for other drugs refer to the salts mentioned.

All drugs were dissolved in 0.9 % saline. The drugs acting on the rectal temperature were used in the following concentrations: histamine 0.5 mg/ml, 2- β -aminoethyl pyridine 8 mg/ml and 3- β -aminoethyl-1, 2, 4-triazole 0.5 mg/ml. The smallest dose used was injected in a volume of 0.1 ml per 100 g of body weight. Increases in dosage were effected by increasing the volume of solution injected.

The injections were made subcutaneously as described previously (WESTLING 1956). Histamine, 2- β -aminoethyl pyridine and 3- β -aminoethyl-1, 2, 4-triazole were injected in the dorsal midline between the shoulder blades, the other substances in the flank.

Plan of experiments. The experiments in tables 1, 2, 3 and 4 were performed after 4 \times 4 Latin square designs. Each of four guinea-pigs was subjected to four different experimental treatments with weekly intervals. The results were subjected to the analysis of variance as described by EMMENS (1948) and SCHILD (1942). The figures for standard deviation (S.D.) given in the heads of tables 1—4 were obtained by taking the square root of the "error" mean square in the analysis of variance.

When responses to only two treatments were compared in a group of guinea-pigs (tables 5 and 6) half of the animals received one treatment (*i. e.* saline and temperature-decreasing drug) in the first experiment and the other treatment (histaminase inhibitor and temperature-decreasing drug) in the second experiment. In the other animals the reverse procedure was adopted. Thus the number of observations on each of the treatments was equal to the number of animals used. Mean values \pm S.E.M. were calculated and compared by the "Student's" *t*-test.

Differences were regarded as statistically significant when the probability (*P*) for them to be caused by chance was less than 0.05. The statistical tables of FISHER and YATES (1953) were used.

Results.

1. *Histamine.* The effect of different doses of histamine on the rectal temperature in a group of four guinea-pigs is shown in table 1 and fig. 3. The changes in temperature

* 2- β -aminoethyl pyridine and 3- β -aminoethyl-1, 2, 4-triazole were made available through the courtesy of dr Reuben G. Jones, Eli Lilly Research Laboratories, Indianapolis, Indiana U.S.A. B₁-pyrimidine was generously supplied by F. Hoffman-La Roche & Co AG, Basel, Switzerland.

Table 1.

Effect of 4 doses of histamine on rectal temperature in 4 male guinea-pigs. The guinea-pigs received the doses in four experiments (numbered I—IV) with weekly intervals. Maximal fall ($^{\circ}$ C) and duration (min.) of the response are given. S.D. for maximal fall 0.65, for duration 30.

Animal no.	Dose of histamine (mg/kg)			
	0.50	0.71	1.00	1.41
1	IV: 0.5° 60 min.	III: 0.7° 70 min.	II: 1.5° 100 min.	I: 3.5° 190 min.
2	II: 1.1° 80 "	IV: 2.1° 140 "	I: 1.9° 120 "	III: 2.9° 190 "
3	I: 1.1° 120 "	II: 1.7° 140 "	III: 3.7° 240 "	IV: 4.3° 220 "
4	III: 1.2° 100 "	I: 1.2° 120 "	IV: 1.6° 120 "	II: 3.0° 150 "
Mean:	0.98° 90 "	1.43° 118 "	2.18° 145 "	3.43° 188 "

Table 2.

Effect of aminoguanidine on the fall in rectal temperature induced by histamine. 4 guinea-pigs. Experiment performed after a 4 × 4 Latin square design. Mean values are given for the four observations on each treatment. S.D. for maximal fall 0.50; for duration 14.

Dose of histamine (mg/kg)	Preceding injection	Maximal fall ($^{\circ}$ C)	Duration (min.)
0.5	Saline	0.50	88
1.0	"	1.50	125
0.5	Aminoguanidine 1mg/kg	1.60	115
1.0	" "	2.48	243

Analysis of variance.

Source of variation	Maximal fall				Duration			
	De-grees of free-dom	Mean Square	Ratio	P	De-grees of free-dom	Mean Square	Ratio	P
Aminoguanidine-saline	1	4.31	16.95	<0.01	1	21025	100.92	<0.001
Regression	1	3.52	13.84	<0.01	1	27225	130.68	<0.001
Parallelism	1	0.02	—	—	1	8100	38.88	<0.001
Animals	3	0.16	—	—	3	3117	14.96	<0.01
Experimental days	3	0.22	—	—	3	317	1.52	>0.2
Error	6	0.25	—	—	6	203	—	—

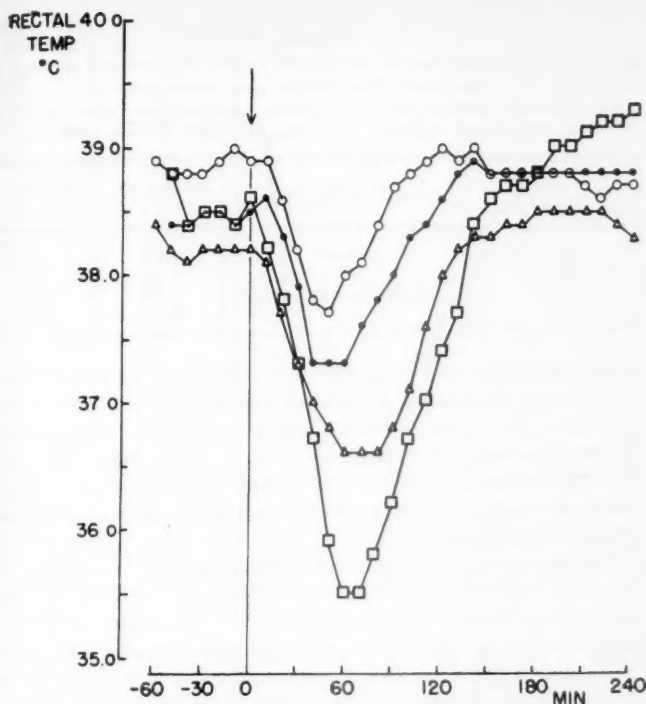


Fig. 1. Effect of different doses of histamine on rectal temperature. 4 experiments on one guinea-pig. Ordinate: Rectal temperature ($^{\circ}\text{C}$). Abseissa: Time in min. before and after injection of histamine (at "0").

○	0.50 mg/kg of histamine.
●	0.71 " " "
△	1.00 " " "
□	1.41 " " "

observed in one animal in four experiments with different doses of histamine are given in fig. 1. Analysis of variance of the results in table 1 revealed a significant linear regression of both the maximal fall and the duration of the response on the logarithm of the dose of histamine ($P < 0.01$). There was no significant deviation from linear regression. There was no significant difference between the responses of the animals or between results from the four experimental days.

The effect of two histaminase inhibitors on the temperature response to histamine was studied. The inhibitors used were

Table 3.

Effect of B₁-pyrimidine on the fall in rectal temperature induced by histamine. 4 guinea-pigs. Experiment performed after a 4 × 4 Latin square design. Mean values are given for the four observations on each treatment. S.D. for maximal fall 0.43; for duration 14.

Dose of histamine (mg/kg)	Preceding injection	Maximal fall (° C)	Duration (min.)
0.5	Saline	1.13	93
1.0	"	1.95	130
0.5	B ₁ -pyrimidine 10 mg/kg	1.90	130
1.0	" "	3.53	218

aminoguanidine and B₁-pyrimidine. These two substances have a powerful inhibitory action on histaminase (SCHULER 1952 and ARUNLAKSHANA, MONGAR and SCHILD 1954, respectively). It was observed previously (WESTLING 1956) that with these two histaminase inhibitors there was a wide margin between doses producing potentiation of histamine effects and doses giving untoward reactions.

A subcutaneous injection of aminoguanidine (1 mg/kg) was found to increase the effect of a subsequent injection of histamine on the rectal temperature (fig. 2). The effect on the rectal temperature of two doses of histamine preceded by aminoguanidine or by a control injection of saline are given in table 2 and fig. 3. It may be seen from the table that both the maximal fall in temperature and the duration of the response were increased. The observed difference in response between experiments with and without aminoguanidine were significant ($P < 0.01$ for maximal fall; $P < 0.001$ for duration). The regression lines for maximal fall with and without aminoguanidine showed no significant deviation from parallelism, whereas with the figures for duration there was a significant deviation ($P < 0.001$). The animals in which the effect of aminoguanidine was studied were somewhat less sensitive to histamine than the groups represented in tables 1 and 3. This may be due to the fact that the experiments with aminoguanidine were performed in July, whereas those of tables 1 and 3 were made in September and October.

An injection of B₁-pyrimidine (10 mg/kg) increased the effect on the rectal temperature of a subsequent injection of histamine (table 3, fig. 3). It may be seen that the action of

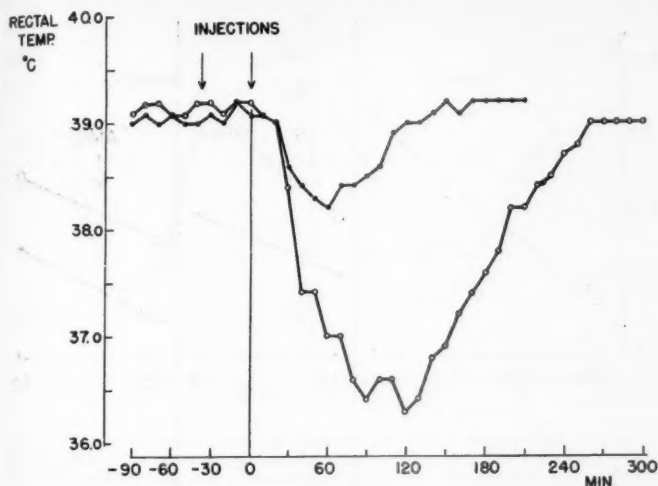


Fig. 2. Potentiation by aminoguanidine of the temperature response to histamine. 2 experiments on one guinea-pig. Ordinate and abscissa as in fig. 1. The right arrow under "Injections" denotes the injection of 1 mg/kg of histamine. The left arrow indicates the injection of saline (curve with filled circles) or 1 mg/kg of aminoguanidine (curve with open circles).

10 mg/kg of B_1 -pyrimidine was similar to that of 1 mg/kg of aminoguanidine. The differences between the responses to histamine with and without B_1 -pyrimidine were significant ($P < 0.01$ for maximal fall; $P < 0.001$ for duration). For the maximal fall there was no significant deviation from parallelism between the regression lines with and without B_1 -pyrimidine but the regression lines for duration showed a significant deviation from parallelism ($P < 0.05$).

2. 2- β -aminoethyl pyridine. 2- β -aminoethyl pyridine has pharmacological actions which are very similar to those of histamine (WALTER, HUNT and FOSBINDER 1941, LEE and JONES 1949, ARUNLAKSHANA, MONGAR and SCHILD 1954). It is, however, not inactivated by histaminase preparations (ARUNLAKSHANA et al. 1954, LINDELL and WESTLING 1957), and in keeping with this, its histamine-like actions have not been potentiated by histaminase inhibitors in different types of experiments (ARUNLAKSHANA et al. 1954, LINDELL and WESTLING 1956, WESTLING 1956).

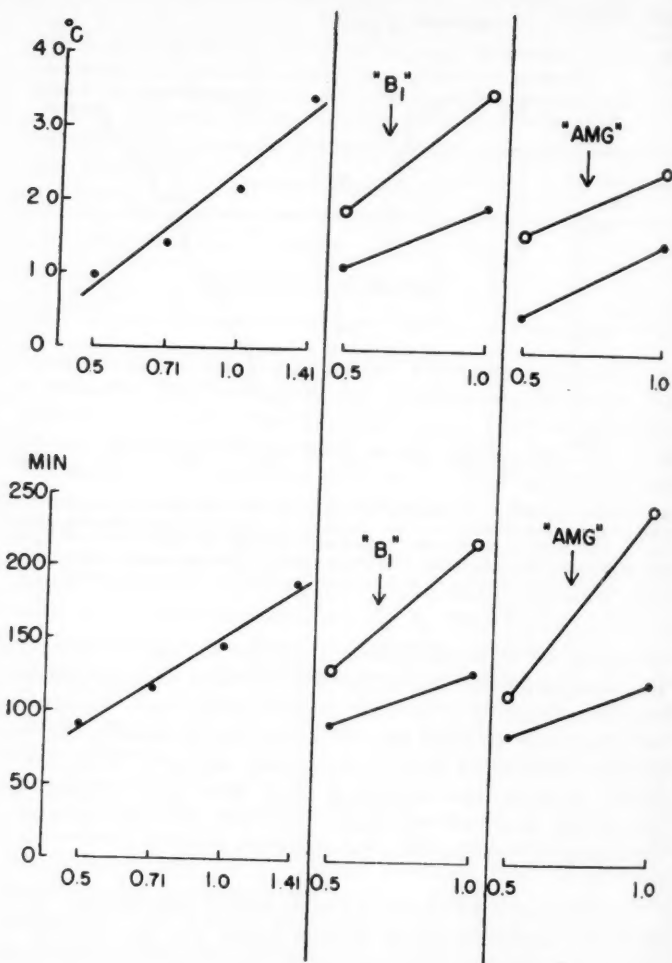


Fig. 3. Comparison of the effects on the rectal temperature of different doses of histamine without and with aminoguanidine or B₁-pyrimidine. The upper half of the figure gives the maximal fall in rectal temperature (°C); the lower half the duration of the response (min.). Doses of histamine are given below the horizontal lines on a logarithmic scale. Results from the three groups of animals of tables 1, 3 and 2 are given from left to right, respectively. The lines with open circles marked "B₁" and "AMG" denote the results obtained after injections of 10 mg/kg of B₁-pyrimidine and 1 mg/kg of aminoguanidine, respectively.

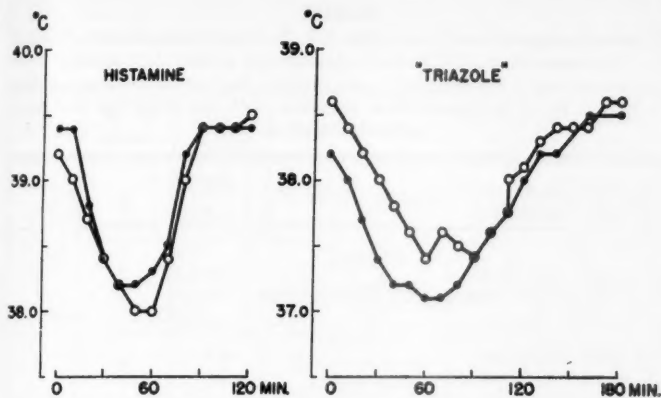


Fig. 4. Effect of mepyramine on the fall in rectal temperature caused by histamine and 3- β -aminoethyl-1, 2, 4-triazole ("triazole"). Each diagram shows two experiments on one guinea-pig. Ordinates: Rectal temperature ($^{\circ}$ C). Abscissas: Time in min. after injection of histamine or "triazole".
 —●— 0.5 mg/kg of histamine or "triazole".
 —○— 2.0 mg/kg of histamine or "triazole", given 30 min. after 1 mg/kg of mepyramine.

2- β -aminoethyl pyridine was found to cause a fall in rectal temperature. In a few experiments the influence of mepyramine was studied. Mepyramine (1 mg/kg), given 30 min. before diminished the sensitivity to 2- β -aminoethyl pyridine so that 32 mg/kg gave about the same or even a smaller response than 8 mg/kg given without mepyramine. Mepyramine caused a reduction of histamine sensitivity of about the same magnitude (fig. 4).

The results obtained with two doses of 2- β -aminoethyl pyridine preceded by saline or aminoguanidine are shown in table 4. One guinea-pig injected with aminoguanidine and the larger dose of 2- β -aminoethyl pyridine died. Otherwise there was no significant difference between the responses to 2- β -aminoethyl pyridine in the experiments with and without aminoguanidine.

The effect of B₁-pyrimidine on the fall in rectal temperature induced by 2- β -aminoethyl pyridine is shown in table 5. The differences between responses in experiments with and without B₁-pyrimidine were not significant.

Table 4.

Effect of aminoguanidine on the fall in rectal temperature induced by 2- β -aminoethyl pyridine. 4 experiments with each of the four treatments on 4 guinea-pigs. 1 guinea-pig died after aminoguanidine + 16 mg/kg of 2- β -aminoethyl pyridine. S.D. for maximal fall 0.84; for duration 16.

Dose of 2- β -aminoethyl pyridine (mg/kg)	Preceding injection	Maximal fall ($^{\circ}$ C) (Mean)	Duration (min.) (Mean)
8	Saline	0.78	88
16	"	2.00	123
8	Aminoguanidine 10 mg/kg	0.75	83
16	"	2.03*	160*

* 3 observations

Table 5.

Effect of B₁-pyrimidine on the fall in rectal temperature induced by 2- β -aminoethyl pyridine. 6 guinea-pigs. Mean values \pm S.E.M. for 6 exp. without and 6 exp. with B₁-pyrimidine are given.

Dose of 2- β -aminoethyl pyridine (mg/kg)	Preceding injection	Maximal fall ($^{\circ}$ C)	Duration (min.)
8	Saline	1.08 \pm 0.18	100 \pm 17
8	B ₁ -pyrimidine 10 mg/kg	1.28 \pm 0.42	103 \pm 22

3. 3- β -aminoethyl-1, 2, 4-triazole. 3- β -aminoethyl-1, 2, 4-triazole has pharmacological actions similar to those of histamine (AINSWORTH and JONES 1953). The triazole compound was not oxidized by histaminase preparations (LINDELL and WESTLING 1957).

It was found that 3- β -aminoethyl-1, 2, 4-triazole, when injected subcutaneously into guinea-pigs, evoked changes in the respiratory movements which were very similar to those seen after an injection of histamine. Injections of 3.5 mg/kg nearly always killed the animal. At autopsy the typical picture of histamine poisoning was seen, e. g. pale, protruding lungs which did not collapse.

3- β -aminoethyl-1, 2, 4-triazole also caused a fall in the rectal temperature. The temperature-reducing effect of the triazole compound was antagonized by mepyramine (1 mg/kg) in about the same degree as was that of histamine, so that about

Table 6.

Effect of 3- β -aminoethyl-1, 2, 4-triazole on rectal temperature in 3 groups of guinea-pigs. Mean values \pm S.E.M. are given.

Group	No. of animals	Dose of "triazole" (mg/kg)	Preceding injection	Maximal fall ($^{\circ}$ C)	Duration (min.)
1	6	0.5	—	1.03 ± 0.18	120 ± 23
		1.0	—	2.02 ± 0.22	188 ± 27
2	6	0.5	Saline	0.85 ± 0.09	106 ± 11
		0.5	Aminoguanidine 10 mg/kg	0.97 ± 0.20	108 ± 9
3	8	0.5	Saline	1.05 ± 0.16	114 ± 14
		0.5	B ₁ -pyrimidine 10 mg/kg	0.91 ± 0.12	110 ± 8

four times more of either drug had to be given to obtain the same effect as without mepyramine (fig. 4).

The fall in rectal temperature elicited by 3- β -aminoethyl-1, 2, 4-triazole was not significantly modified by aminoguanidine or B₁-pyrimidine (table 6).

4. Effect of large doses of aminoguanidine and B₁-pyrimidine on rectal temperature. Aminoguanidine alone had no effect on the rectal temperature, when injected subcutaneously in a dose of 10 mg/kg. Four guinea-pigs were injected with 100 mg/kg of aminoguanidine. In two animals a slight increase in the temperature was observed. The biggest rise was 0.8° C. In the other two guinea-pigs a distinct effect could not be seen.

B₁-pyrimidine, injected subcutaneously into 4 guinea-pigs in a dose of 100 mg/kg, did not cause any change in rectal temperature.

Discussion

The earlier observation, that the effects of subcutaneously injected histamine on unanaesthetized guinea-pigs were potentiated by histaminase inhibitors (WESTLING 1956), has been extended in this investigation. In the earlier experiments the duration of changes in the external respiratory movements

after histamine was studied. In the present experiments another reaction to histamine was used, namely the fall in rectal temperature. This reaction lends itself more readily to quantitative analysis. The increase of the effect of histamine on the rectal temperature after injection of 1 mg/kg of aminoguanidine or 10 mg/kg of B₁-pyrimidine was about two-fold in the present experiments. A similar degree of potentiation was observed in the previous studies of the respiratory changes.

The augmentation by aminoguanidine and B₁-pyrimidine of the effect of histamine on the rectal temperature was not an additive effect since it was obtained with doses of histaminase inhibitors that did not cause changes in the rectal temperature when given alone. Even doses of 100 mg/kg of the inhibitors had no temperature-decreasing effect.

The effects of 2- β -aminoethyl pyridine and 3- β -aminoethyl-1, 2, 4-triazole on the rectal temperature were also studied. These substances belong to a group of so called "histamine analogues". Histamine analogues have pharmacological actions that are very similar to those of histamine and their actions are antagonized by antihistamines to the same extent as those of histamine, indicating that they act on the same "receptors" as histamine (ARUNLAKSHANA, MONGAR and SCHILD, 1954). The present experiments showed that 2- β -aminoethyl pyridine and 3- β -aminoethyl-1, 2, 4-triazole decreased the rectal temperature of guinea-pigs and that this effect was antagonized by mepyramine. A direct comparison of the relative effects of histamine and the two histamine analogues in the same animal was not made. However, on the basis of experiments performed under uniform conditions on guinea-pigs of roughly the same body weight, it may be said that the ratio between equiactive doses of histamine, 2- β -aminoethyl pyridine and 3- β -aminoethyl-1, 2, 4-triazole was about 1:16:1.

As mentioned above, neither 2- β -aminoethyl pyridine nor 3- β -aminoethyl-1, 2, 4-triazole are oxidized by histaminase preparations *in vitro*. In agreement with this is the present finding that the effects of these two histamine-like substances on the rectal temperature were not increased by aminoguanidine or B₁-pyrimidine. This observation supports the assumption that the potentiation by aminoguanidine and B₁-pyrimidine of the effects of subcutaneously injected histamine on unanaesthetized guinea-pigs is caused by an inhibition of histaminase and not by other actions of the inhibitors.

Summary

1. The effects of subcutaneous injections of histamine on the rectal temperature of guinea-pigs were potentiated by aminoguanidine and 2-methyl-4-amino-5-methylaminopyrimidine, two histaminase inhibitors.

2. The histaminase inhibitors did not increase the response of the rectal temperature to two "histamine analogues", which have pharmacological effects resembling those of histamine. The "histamine analogues" used are resistant to histaminase in vitro.

I am grateful to dr. H. O. SCHILD for advice on the statistical treatment of the data. The technical assistance given by Miss M.-B. JOHANSSON was highly appreciated.

This investigation was aided by a grant from the Medical Faculty, University of Lund.

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Histamine as a Stimulant to the Anterior Pituitary Gland as Judged by the Lymphopenic Response in Normal and Hypophysectomized Rabbits.

By

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Received 27 February, 1957.

It is well established that damage to the body or its component cells as well as various adverse conditions may cause a liberation of histamine. Even lesser events, not entailing gross cell damage, such as allergic reactions in man, asphyxia or elevation of the concentration of adrenaline in plasma, are thought to induce a liberation of histamine into the blood stream. Because of limitations in technique and methods so far available it is not known just what minimum assault to homeostasis or to the integrity of cells is required to shift histamine from the intracellular into the extrinsic state. It is, however, well known that conditions and procedures effective in liberating histamine will concomitantly cause increased activity in the pituitary-adrenal system. This coincidence prompts the question whether liberated, extrinsic, blood carried histamine may play a part in stimulating the pituitary-adrenal system.

There are several experiments on record, mainly on rats, showing that injections of histamine are followed by a reduction in adrenal ascorbic acid content and a fall in the number of blood eosinophils. References to literature are given, amongst others, by FORTIER 1952, GUILLEMIN 1955, SWINGLE, EISLER, BAKER, LE BRIE and BRANNICK 1955. The doses of histamine used in these experiments on rats, milligrams and hundreds of

milligrams per kilogram body weight, exceed by powers the amounts which conceivably could be liberated under natural conditions and therefore add little information to the pertinent problem. Only a few observations with "physiological" quantities of histamine have so far been published. KULLANDER (1952) noted in pregnant women that 10 $\mu\text{g/kg}$ histamine subcutaneously was followed by an eosinopenia and ALEXANDER and ASH (1955) observed in the horse that 10—20 $\mu\text{g/kg}$ histamine intravenously produced eosinopenia. In these two sets of experiments the effect of histamine after hypophysectomy was not investigated.

For the purpose of the present study the eosinopenic response was not chosen as an indicator of enhanced pituitary-adrenal activity since it has been demonstrated that in the dog lacking both adrenal glands intravenous injection of histamine causes a sharp decline in the number of circulating eosinophils (SWINGLE et al. 1955). The ascorbic acid content of the adrenal glands was also an unsuitable index in the present study because it seemed desirable to make several observations in one and the same animal.

The lymphopenic response in unanaesthetized rabbits has been found of great value as an indicator of anterior pituitary activity (COLFER, DE GROOT and HARRIS 1950, DE GROOT and HARRIS 1950). In the present study we investigated in rabbits the lymphopenic response to histamine concentrations which are likely to fall within the range of those occurring naturally.

Methods.

Care of the animals. Adult rabbits of both sexes, weighing 2—3.5 kg. were used. Living conditions and treatment were kept as constant as possible throughout. The diet consisted of oats, roots, greenstuff, as available, and the temperature of the animal quarters was maintained at a fairly constant level. The animals were accustomed to handling and to the laboratory surroundings, and were brought into the laboratory on the day before any experiment in order to avoid incidental discomfort at the commencement of the experiment, which was performed under uniform, quiet conditions.

Blood sampling and counting. Blood samples were taken from the marginal ear-vein, care being taken to avoid squeezing or rubbing, and only freely flowing blood being used. Where possible one ear was reserved for injections, the other for blood sampling. Total white blood counts were made using a Bürker chamber, the blood being diluted 1 : 20 with 1 % acetic acid coloured with gentian violet. The number of cells in a volume of 0.2 cu. mm of diluted blood was counted,

i.e. in 50 small squares on the chamber, each such square having a volume of 1/250 cu. mm. Triplicate blood samples were taken, the third being counted only when the first two did not agree. Differential blood counts were made upon smears stained with May-Grünwald-Giemsa solutions. On each smear 300 white blood cells were counted, and distinction was made between lymphocytes, monocytes and granulocytes. For all blood counts a hand-operated electrical recording device was used.

Operations. Hypophysectomy was performed by the parapharyngeal route (JACOBSON and WESTMAN 1940) and the completeness of the removal was controlled by microscopic examination of serial sections, 10 μ thick and stained with haematoxylin-eosin, through the contents of and the tissues above the sella turcica. These operations as well as the final microscopic control were made by Dr. DORA JACOBSON.

In some highly excitable animals removal of the superior cervical ganglia in the neck, and the great auricular nerves at the base of the ear facilitated injection and blood sampling. Demedullation of the adrenal glands was achieved using thermocautery, the gland being split and the medulla destroyed.

Emotional stimulation. This was effected by passing a faradic current of frequency 50 per second and a pulse duration of 10 milliseconds, at 33 volts for a period of 2 minutes, through two electrodes, one inserted subcutaneously over the lumbar vertebrae and the other into the rectum. The strength of the stimulus was such as to elicit tachycardia, polypnoea and signs of emotional disturbance, struggling and screaming. Although individual rabbits behaved differently to this stimulus, repeated stimulation of one and the same rabbit evoked a rather uniform pattern of behaviour.

Injections. All intravenous injections were made into the marginal ear-vein. The volume of all injections was 1.0 ml and the drugs dissolved in 0.9 % warm (w/v) NaCl solution. The duration of the injection was 2 minutes.

Drugs. Two preparations of adrenocorticotrophic hormone were used, ACTH Armour and an ACTH protein donated by Dr. C. H. Li. Histamine acid phosphate was used and the doses expressed in terms of the base. Solutions of adrenaline and nor-adrenaline were made up freshly from the powdered hydrochloride and the 1-arterenol bitartrate monohydrate (Sterling-Winthrop). Neoantergan (mepyramine) Merck and pyribenzamine HCl Ciba were also used.

Results.

The experiments were done in all seasons during the years 1953—1956. Observations were made on a total of twenty rabbits. The responsiveness of the anterior pituitary gland and of the adrenal cortex, respectively, was tested at suitable times by emotional stimulation and injection of ACTH. Emotional stimulation has been shown to produce a lymphopenia in the intact

but not in the hypophysectomized rabbit (COLFER, DE GROOT and HARRIS 1950). Responses to injections of drugs were considered significant only in animals and at periods where a control injection of 1 ml body warm saline caused a change in the number of blood lymphocytes not greater than $\pm 5\%$. Blood samples for counting were taken at one, two and three hours after injection or emotional stimulation. The lymphopenic response is expressed as percent change in relation to the initial number. There was an interval of at least one day between tests in one and the same animal.

ACTH. In order to test the responsiveness of the composite peripheral mechanism, the activation of which results in a drop in blood lymphocytes, ACTH was injected at various phases of the investigation. On intravenous injection 0.05 mg/kg ACTH gave definite lymphopenic responses. The materials prepared by ARMOUR and by Dr. LI were about equiactive in our observations. The results of experiments with ACTH in sixteen rabbits are given in Table 1. From the figures it will be seen that among the group of rabbits, and even in one and the same individual, the responsiveness to an equal dose of ACTH may vary considerably. For this reason it can not be expected that injection of a substance augmenting the release of ACTH should give consistent lymphopenic responses. This is well illustrated in rabbit no. 15 where 0.025 mg/kg ACTH in test no. 3 gave a definite response while the same dose two days later failed to do so. For this reason distinct lymphopenic responses appear to be more significant of stimulating potency than occasional failures, especially when the stimulant is applied at a level around the threshold dose.

With ACTH Armour the maximum drop in five cases out of ten had been reached at 1 hour after injection. With Dr. LI's ACTH protein the lymphopenia was greatest at 1 hour in four experiments out of nine.

Following injections of ACTH changes in the number of granulocytes occurred which even in one and the same animal were rather irregular. A granulocytosis was the most frequent result. The figures in the tables denote the maximum change in the number of granulocytes observed during 3 hours.

Emotional stimulation. This was done in order to test the responsiveness of the anterior pituitary gland to a type of stimulation known to increase the discharge of ACTH as indicated by the lymphopenic response (COLFER, DE GROOT and HARRIS 1950).

Table 1.

Lymphopenic response at 1, 2, and 3 hours following injection of ACTH, expressed as per cent change from initial 100 %. The Armour preparation was used except in experiments marked L, where Dr. LI's protein was injected. Changes in the number of granulocytes in per cent are given in the last column.

Rabbit no. and sex	Operation Exp. no.	Dose in mg/kg	at 1	at 2 hours	at 3	Granulocytes
3 ♂	2	0.05 L	— 30	— — 45	—	+ 75
11 ♂	11	0.05	— 30	— —	—	— 10
12 ♂	1	0.1 L	— 61	— 75	— 64	+ 450
	9	0.1	— 39	— —	—	+ 400
	10	0.1	— 37	— —	—	+ 20
15 ♀	1	0.03	— 60	— 52	— 47	+ 12
	2	0.015	— 17	— 21	— 47	+ 50
	3	0.025	— 17	— —	— 32	+ 32
	4	0.025	— 0	— 0	—	— 35
	6	0.03 L	— 24	— 24	—	0
	7	0.03	— 38	— —	—	— 18
	14	0.03	— 13	— —	—	+ 275
	16	0.03	— 38	— —	—	0
	17	0.03	— 11	— —	—	—
18 ♀	1	0.1 L	— 35	— 31	— 10	+ 6
	2	0.1	— 38	— 36	— 0	+ 460
	10	0.1	— 43	— —	—	— 20
	11	0.1	— 52	— —	—	— 26
	16	0.1	— 15	— —	—	+ 32
	18	0.1	— 41	— —	—	— 56
	23	0.1	— 29	— —	—	— 54
	24	0.1	— 26	— —	—	0
19 ♂	1	0.1	— 62	— 55	— 30	+ 250
21 ♀	1	0.1 L	— 14	— 27	— 0	0
	2	0.1	— 65	— 24	— 0	+ 400
	8	0.1	— 41	— —	—	+ 16
	9	0.1	— 58	— —	—	+ 42
	13	Sham	— 31	— —	—	+ 60
	16	Hyp.ect.	— 27	— 34	—	+ 220
	17	0.1	— 40	— —	—	— 18
	17	0.1	— 40	— —	—	— 18
22 ♀	2	0.1	— 31	— —	—	— 20
	3	0.1	— 37	— —	—	0
	22	0.05	— 49	— 50	— 41	0
	28	0.05	— 29	— 39	— 40	— 20

Table 1.

continued

Rabbit no. and sex	Operation Exp. no.	Dose in mg/kg	at 1	at 2 hours	at 3	Granulo- cytes
23 ♂	2	0.1	— 36	—	—	+ 46
	3	0.1	— 17	—	—	0
24 ♀	2	0.1	— 32	—	—	— 25
	3	0.1	— 34	—	—	+ 44
25 ♂	2	0.05	— 32	—	—	+ 48
	13	0.05	— 41	—	—	+ 56
26 ♂	2	0.05	— 30	—	—	0
	9	0.05	— 33	— 32	— 9	+ 280
27 ♀	3	0.05	— 39	— 27	— 14	+ 20
31 ♀	3	0.05	— 32	— 13	0	0
	10	0.05	— 29	— 22	— 21	+ 12
32 ♀	4	0.05	— 22	— 36	— 18	0
	10	0.05	— 32	—	—	+ 220
89 ♀	Demedull. 4	0.05	— 23	— 37	— 14	+ 50

The agent engaged in the process of stimulation appears to be humoral, carried presumably from the brain to the pituitary gland by the hypophyseal portal vessels (evidence reviewed by HARRIS 1955, FORTIER 1956). HARRIS and his associates (1950) have shown that emotional stimulation in hypophysectomized rabbits does not produce any lymphopenia, a fact which, in addition, provides useful control of the completeness of the hypophysectomy. Further, it seemed of interest to compare, in individual rabbits, the pattern of response to intravenously injected histamine and to emotional stimulation. Observations on 12 rabbits are presented in Table 2. Emotional stimulation regularly caused a transient lymphopenia in the intact rabbit. In some cases the response was maximal at the first hour, in others at the second or third hour after stimulation. The response to emotional stimulation is rather variable in magnitude and shape, which also applies to the response to ACTH. On comparing

Table 2.
Effects of emotional stimulation.

Rabbit no. and sex	Operation Exp. no.	at 1	at 2 hours	at 3	Granulocytes maxim. change
9 ♂		-44 -46	-34 -39	-22 -21	+300 +500
12 ♂	Hyp.ect. 6 7 8 11 12	-32 -28 -33 -9 -7	-31 -28 -51 — —	-20 -40 -51 — —	+82 +220 +310 +10 +100
15 ♀	10 15 18	-43 -19 -44	— — —	— — —	+10 +58 -36
18 ♀	Hyp.ect. 8 9 13 14 25 26	-29 -28 -13 -14 -34 -22	-44 -48 — — — —	-49 -19 — — — —	+57 0 +22 +5 +20 +9
21 ♀	{ Sham Hyp.ect. 6 7 10 11	-28 -42 -41 -40	-33 -39 -28 —	-21 — -41 —	+268 +63 +80 +26
22 ♀	Hyp.ect. 1 16 21 Demedull. 27	-26 -13 -6 +6	— -17 -5 +8	— -11 -5 +18	0 -18 +25 -52
23 ♂	Hyp.ect. 1 23 26	-20 -8 -2	— -13 -11	— -6 -5	-18 +32 +3
24 ♀	Hyp.ect. 1 18 19	-26 -16 -20	— -18 -22	— -10 -12	+3 +23 0

Table 2.

continued

Rabbit no. and sex	Operation Exp. no.	at 1	at 2 hours	at 3	Gramulocytes maxim. change
31 ♀	Demedull. 1	- 37	- 33	- 9	0
	14	- 10	+ 20	+ 10	+ 68
	17	- 25	- 10	- 4	+ 90
	29	- 28	- 2	+ 4	0
32 ♀	Demedull. 1	- 26	- 8	- 2	+ 20
	9	- 10	- 13	- 2	+ 55
	14	+ 5	- 24	- 4	0
	16	- 20	- 24	- 15	+ 50
	28	- 23	- 20	- 8	+ 32
33 ♂	Hyp.ect. 2	- 5	- 4	- 3	+ 18
89 ♀	Demedull. 1	- 11	- 13	+ 30	+ 25
	7	- 13	- 2	- 1	0
	10	- 22	- 14	- 5	0
	21	- 32	- 8	- 6	+ 58

the responses to ACTH and to emotional stimulation in the individual rabbits, presented in Table 1 and 2, no obvious parallelism in the responses to the two types of stimulation will be seen. Such parallelism is hardly to be expected because emotional stimulation induces, in addition to the discharge of ACTH, the liberation of adrenaline, nor-adrenaline, and possibly other agents interfering with the lymphopenic response.

After complete removal of the pituitary gland emotional stimulation does not cause the typical transient lymphopenia. Rabbits no. 18, 21 and 24 deserve comments. In rabbit no. 18 emotional stimulation 15 and 18 days after hypophysectomy failed to induce distinct lymphopenia (13 and 14 % drop at 1 hour) but was effective in causing this response two months after the operation (34 and 22 % drop at 1 hour). Post mortem examination revealed a minute hypophyseal remnant. The weight of the ovaries was 67 + 69 mg and that of the adrenals 150 + 125 mg, indicating moderate atrophy only. The return of the response to emotional stimulation in this rabbit is probably related to regeneration of vascular connections between the tuber

cinereum and retained fragments of pituitary tissue as described by COLFER, DE GROOT and HARRIS 1950. The response to injected histamine in this rabbit will be discussed in a following paragraph.

In rabbit no. 21 a sham operation for hypophysectomy was performed. From the third week onwards after the operation emotional stimulation gave definite responses (41 and 40 % drop at 1 hour). In rabbit no. 24 a slight but distinct response occurred even after complete hypophysectomy. This rabbit showed lymphopenic response to injection of small doses of adrenaline as will be seen in Table 5. Confirming COLFER, DE GROOT and HARRIS following emotional stimulation a granulocytosis was noted in the majority of cases.

Emotional stimulation after demedullation. In rabbits no. 31, 32 and 89 the adrenal medulla was destroyed by cauterization. The process of cortical regeneration and functional recovery can easily be assessed by the gradual return of the lymphopenic response to injection of ACTH. It will be seen from Table 2 that in these rabbits distinct lymphopenic responses can be induced by emotional stimulation and that the magnitude of the response increases with the length of time after demedullation. Obviously, medullary adrenaline is not an essential part in the lymphopenic response to emotional stimulation. Again, this confirms COLFER, DE GROOT and HARRIS who found that denervation of the adrenal glands did not alter the lymphopenic response to this type of stimulation.

Injection of saline, significance of response. In the rabbits referred to in the various tables 1 ml 0.9 % NaCl was injected during 2 minutes in twenty six cases. The mean values obtained for changes in the number of lymphocytes at 1, 2 and 3 hours, respectively, are as follows: — 3.7 ± 0.6 % (26), — 2.9 ± 0.53 % (20), + 0.9 ± 0.5 % (18). It appears safe to consider a decrease in lymphocyte count of 15 % or more as significant.

Effect of hypophysectomy on lymphocyte count. Observations on four rabbits are summarized in fig. 1. From the second week after the operation onwards the fluctuations occurring in counts made on one and the same rabbit are not greater than in the intact group nor is there any obvious difference in the total number of lymphocytes between hypophysectomized and normal rabbits. This information is given to underline that in the experiments now to be described the two groups of rabbits are fully comparable as regards pre-injection lymphocyte level.

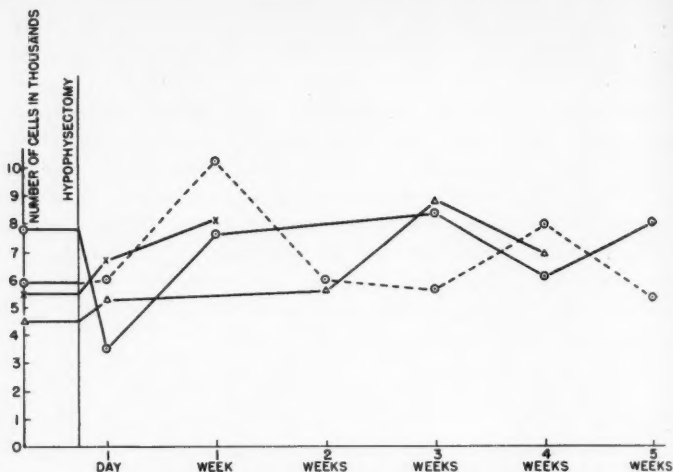


Fig. 1. Levels of blood lymphocytes before and after hypophysectomy in four rabbits.

Histamine. As already mentioned it was planned in the present investigation to use doses of histamine which in magnitude are within the quantities known to be liberated under natural conditions. We noted that 25 $\mu\text{g}/\text{kg}$ histamine, in terms of the base, injected intravenously in two minutes, was the smallest dose to give a slight lymphopenic response in most cases. In its effect this dose was roughly comparable to 0.025 mg/kg ACTH. It was therefore decided to use 50 $\mu\text{g}/\text{kg}$ histamine as a standard dose, and 100 $\mu\text{g}/\text{kg}$ for additional information. These doses caused no visible changes in the animal. Such doses are likely to augment the discharge of adrenaline from the adrenal medulla (for literature see EMMELIN and MUREN 1949). Adrenaline, in turn, induces a lymphopenic response. For this reason the effect of histamine was investigated, in addition, in three rabbits after destruction of the adrenal medulla. Observations on sixteen rabbits are presented in Table 3. In the intact rabbit intravenous injection of 50 $\mu\text{g}/\text{kg}$ histamine is, with few exceptions, followed by a temporary fall in the number of blood lymphocytes. In exceptional rabbits, where this dose failed to induce the lymphopenic response, the dose was found effective at further trials after intervals of one or a few days. In the individual animal the magnitude of the response to repeated injections of histamine is very

Table 3.
Effects of histamine.

Rabbit no.	Operation Exp. no.	Dose $\mu\text{g/kg}$	at 1	at 2 hours	at 3	Granulo- cytes
5	4	50	-36	-31	-11	-
	7	50	-30	-15	0	-
	9	50	-18	-	-	-
12	3	50	-38	-6	-	+ 230
	4	50	-31	-48	-	+ 350
	13	50	+ 28	-	-	+ 34
15	12	100	-19	-	-	- 12
	13	50	-21	-	-	- 23
	19	50	-37	-	-	+ 9
18	3	50	-32	-27	-32	- 22
	15	50	+ 7	-	-	- 10
	17	50	0	-	-	0
	20	50	-20	-	-	- 26
	21	50	-3	-6	-	+ 18
	22	50	-39	-	-	+ 82
19	4	50	-38	-	-	+ 70
21	4	100	-45	-21	-21	+ 23
	12	100	-25	-	-	+ 41
	15	50	-11	-	-	+ 12
	18	100	-13	-	-	- 11
	19	100	-18	-	-	+ 20
22	4	50	-26	-	-	- 9
	9	50	-19	-7	-	- 40
	10	100	-14	-17	-29	+ 42
	17	50	-5	+ 9	-8	+ 30
	18	50	+ 4	-2	-4	0
	23	100	-8	-3	-2	- 10
	24	100	-2	-7	-2	0
	Demedull.	30	-2	-11	-6	0
23	4	50	-6	-	-	- 53
	9	50	+ 1	-	-7	+ 11
	10	100	-16	-26	-23	0
	21	50	-20	-20	-18	+ 28
	24	50	-7	-11	-16	- 10
	25	50	-5	+ 11	-5	+ 25
	28	50	+ 8	-4	-6	0
	34	50	-10	+ 3	-6	0

Table 3.

continued

Rabbit no.	Operation Exp. no.	Dose $\mu\text{g/kg}$	at 1	at 2 hours	at 3	Granulo- cytes
24	7	50	- 29	- 4	0	0
	8	100	- 10	- 7	5	+ 38
	10	50	- 18	- 8	- 7	0
25	5	50	- 12	- 10	-	- 46
	6	100	- 10	-	-	+ 25
	9	50	- 26	- 30	- 12	0
26	5	50	- 21	-	- 11	- 26
	8	50	- 10	- 18	- 9	0
	14	100	- 28	- 26	- 22	- 27
31	4	50	- 17	- 23	- 21	- 10
	7	25	- 11	- 7	- 4	0
	11	50	- 33	- 22	-	+ 300
	15	50	- 38	- 12	- 12	+ 10
	18	50	- 15	- 37	- 23	0
32	5	50	- 23	- 23	- 20	+ 16
	8	50	- 24	- 21	- 7	0
	12	50	-	- 23	- 24	+ 53
	13	50	0	- 15	- 16	0
	15	50	- 33	- 15	- 11	+ 5
	17	50	- 18	- 35	- 30	+ 14
33	Hyp.ect.					
	1	50	+ 3	+ 16	+ 10	+ 26
	5	25	0	+ 3	+ 3	+ 15
89	Demedull.					
	5	50	- 22	- 14	- 5	0
	8	50	- 27	- 20	- 3	+ 65
	12	50	- 7	- 29	- 5	0

variable. This is also true for ACTH. In seventeen of thirty histamine injections where counts were made at 1, 2 and 3 hours the maximal drop occurred at 1 hour. Again, a similar pattern of response was noted with ACTH.

After hypophysectomy injection of histamine fails to give the lymphopenic response (no. 12, 18, 22, 23, 33). In rabbit no. 18, following hypophysectomy, histamine as well as emotional stimulation failed to induce lymphopenia during the first four weeks after the operation. From the sixth week after the operation

onwards histamine as well as emotional stimulation became effective, indicating reactivation and regained responsiveness of a hypophyseal remnant, as commented on in the preceding paragraph on emotional stimulation. Rabbit no. 33 was already hypophysectomized at the beginning of these experiments. In rabbit no. 21 a sham operation for hypophysectomy was performed.

In rabbit no. 22 the adrenal medulla was removed fourteen weeks after hypophysectomy.

Injections of histamine were followed by changes in the number of granulocytes which even in the individual rabbit were entirely irregular. Similar irregular changes were noted with ACTH as seen from Table 2.

Destruction of the adrenal medulla neither abolished nor significantly modified the lymphopenic response to injected histamine (no. 31, 32, 89). It thus appears that the lymphopenic response to histamine is not mediated by liberated adrenaline.

Histamine antagonists. There are several reports on the effect of histamine antagonists on the ascorbic acid depletion of the adrenals and on the eosinopenia induced in rats by enormous doses of histamine (for references see SWINGLE et al. 1955). The observations on record are controversial. Neoantergan (mepyramine), 5 mg/kg, was ineffective in antagonizing the effect of 150 milligram histamine per kg on the adrenal content of ascorbic acid in the rat (NASMYTH 1951), whereas after mepyramine (2.5 mg/kg), which by itself caused a small fall in the adrenal ascorbic acid, 10 milligram histamine per kg did not add to the fall caused by mepyramine alone (NASMYTH 1953). TEPPERMAN, RAKIETEN, BIRMIE and DIERMEIER (1951) report that histamine injections after pretreatment with pyribenzamine give a smaller fall in adrenal ascorbic acid than that in control animals, whereas GUILLEMIN and FORTIER (1953) by ten days of repeated administration of phenergan achieved a complete inhibition of the normal adrenal ascorbic acid discharge in response to histamine injection. HALPERN and BENOS (1952) noted that mepyramine prevented the drop in eosinophils caused by histamine in the dose of 10 milligram per kg in the rat. In dogs SWINGLE et al. (1955) found pyribenzamine (1 mg/kg intravenously) without significant effect on the number of blood eosinophils and ineffective in preventing the profound fall in eosinophils induced by solubilized cortisone.

Table 4.

Effects of histamine antagonists. NA = neoantergan, P = pyribenzamine, E. S. = emotional stimulation. All injections are intravenous except those marked s.c. = subcutaneously. I = interval in minutes between injection of antagonist and histamine.

Rabbit no. Exp. no.		NA or P mg/kg	Histamine μ g/kg	I	at 1	at 2 hours	at 3
6	1	NA 0.3 s.c.	300	60	-31	—	8
	5	NA 1.0	300 s.c.	60	-29	—	29
	7	NA 1.0	300	60	-33	-26	-26
	8	NA 1.0	500	60	-23	-10	-10
	9	NA 1.0	0	—	-27	+ 6	+13
	10	NA 2.0 s.c.	0	—	+10	- 8	+32
	11	NA 2.0 s.c.	300 s.c.	60	-21	-30	-21
	12	NA 1.0 s.c.	500 s.c.	60	-28	-32	-32
8	2	NA 1.0 s.c.	500	60	-58	—	-15
	9	NA 2.0 s.c.	0	—	0	+ 6	0
	10	NA 2.0 s.c.	300 s.c.	60	-29	-12	- 6
22	14	NA 1.0	0	—	-10	+ 1	+ 4
Demedull.							
31	23	NA 1.0	50	60	+ 4	-12	-12
	25	NA 2.0	0	—	-16	-30	-39
	26	NA 1.0	50	15	- 9	-13	-21
	27	NA 1.0	E. S.	15	-41	-27	-20
	28	NA 1.0	0	—	+ 6	-13	+ 1
	30	P 1.0	0	—	+20	+56	+ 6
	31	P 1.0	50	30	- 3	-15	-10
	32	P 1.0	0	—	+ 3	+11	-17
	33	P 1.0	0	—	+ 6	+27	+28
Demedull.							
32	21	NA 1.0	0	—	+ 2	-10	- 2
	22	NA 1.0	50	15	- 9	-22	-15
	23	NA 1.0	0	—	- 3	-12	- 1
	24	NA 1.0	50	15	-23	- 3	- 5
	25	NA 1.0	50	15	-27	-17	-14
	26	NA 1.0	0	—	+ 2	-14	+ 7
	27	NA 1.0	0	—	-18	+ 3	+21
	29	P 1.0	0	—	+22	+15	-19
	30	P 1.0	0	—	+12	- 8	- 3
	31	P 1.0	0	—	- 2	-28	-18
Demedull.							
89	15	NA 1.0	E. S.	15	-38	-16	—
	16	NA 2.0	0	—	+ 5	-30	-16
	17	NA 1.0	0	—	- 8	-13	0
	18	NA 1.0	50	15	- 5	-18	- 9
	19	NA 1.0	E. S.	15	-40	-10	+20
	20	NA 1.0	0	—	-13	- 7	+ 6
	22	P 0.1	0	—	-10	- 6	0
	23	P 1.0	0	—	-22	-17	- 1

Observations on six rabbits are given in Table 4. It may be seen 1) that neoantergan in the dose 1.0 mg/kg does not cause significant lymphopenic responses, 2) that the effect of pyribenzamine on the number of cells is irregular, sometimes resulting in lymphocytosis, sometimes in lymphopenia making it useless in this study, 3) that neoantergan does not abolish the lymphopenic response to histamine or 4) to emotional stimulation.

It may be questioned whether the doses of antihistamine drugs used in the present experiments are adequate to display their inherent antagonistic potency. A definite answer appears difficult because in the rabbit measurable responses to 50 μ g/kg histamine are meagre. The doses of antagonists used appear adequate, firstly because in some cases they by themselves induce effects on the blood cells (Table 4), further, by analogy, because EMMELIN and MUREN (1949) have shown that neoantergan and pyribenzamine in doses as small as 10 μ g abolish the adrenaline liberating effect of 0.6 μ g histamine injected into the coelic artery in the cat.

Adrenaline. Under the influence of emotional stimulation and various offences against homeostasis the adrenal medulla easily liberates adrenaline, which is thought to exert a stimulating effect on the anterior pituitary gland (for references see LONG 1952, HARRIS 1955 and FORTIER 1956). Histamine is also a potent liberator of adrenaline.

COLFER, DE GROOT and HARRIS (1950) investigated the effect of intravenous injection of adrenaline on the blood lymphocytes. In seven normal rabbits weighing 2–3 kg and injected with a total dose of 6.7 μ g, corresponding to 2–3 μ g/kg, these authors obtained a definite lymphopenic response in one case (–24 %), one doubtful response (–13 %) and five negative responses.

Our experiments with adrenaline differ from the above mentioned by using somewhat larger doses, 5 and 10 μ g per kg, by performing the injection more slowly, during 2 minutes, and by including observations on hypophysectomized animals. The results are presented in Table 5 from which it will be seen 1) that adrenaline, 10 μ g/kg, in twenty injections out of twenty-two gave a distinct lymphopenic response, 2) that the smaller dose, 5 μ g/kg, caused this response in five of eleven trials, and 3) that hypophysectomy did not abolish the lymphopenic response to adrenaline. In only eight of twenty-three 3 hours counts did the maximal fall occur at the first hour. This pattern seems to differ from those caused by ACTH and histamine. The changes in

Table 5.
Effects of adrenaline.

Rabbit no.	Operation Exp. no.	Dose $\mu\text{g}/\text{kg}$	at 1	at 2 hours	at 3	Granulo- cytes
22	11	10	- 34	+ 5	+ 3	+ 38
	12	10	- 37	- 28	- 10	+ 84
	13	5	- 3	- 4	- 2	+ 230
	Hyp.ect. 19	5	- 20	- 18	- 26	0
	20	10	- 12	- 17	- 20	- 21
	Demedull. 29	10	- 16	- 10	- 2	+ 14
	36	10	+ 11	- 16	- 9	+ 280
23	14	10	- 14	- 8	- 2	+ 32
	15	5	- 23	- 17	- 10	+ 14
	16	5	- 6	- 7	- 7	- 33
	17	10	- 22	- 2	+ 1	+ 225
	18	5	- 7	- 2	+ 6	- 10
	Hyp.ect. 27	5	- 11	- 22	- 5	+ 26
	29	10	- 7	- 17	- 10	0
	30	5	- 13	- 18	- 25	- 24
	35	10	- 15	- 16	- 16	0
24	12	10	- 33	- 22	- 17	0
	13	5	+ 14	+ 21	+ 12	+ 60
	14	5	- 2	- 14	- 8	+ 14
	15	5	- 14	- 4	- 3	+ 50
	17	10	- 8	- 18	- 7	+ 70
25	11	10	- 10	- 8	- 6	+ 72
	12	5	- 5	- 1	+ 5	- 58
26	10	10	- 33	- 12	- 10	+ 60
	11	5	- 7	- 9	- 11	+ 22
	13	10	- 1	+ 6	+ 9	+ 18
31	Demedull. 5	10	- 10	- 22	- 7	+ 28
	20	10	- 24	- 26	- 17	+ 31
32	Demedull. 6	10	- 18	- 20	- 5	+ 48
33	Hyp.ect. 3	10	- 12	- 22	- 10	+ 26
89	Demedull. 6	10	- 9	- 27	- 18	0
	9	10	- 15	- 16	- 4	+ 82
	13	10	- 10	- 26	- 4	0

Table 6.
Effects of nor-adrenaline.

Rabbit no.	Operation Exp. no.	Dose $\mu\text{g/kg}$	at 1	at 2 hours	at 3	Granulo- cytes
22	Hyp.ect. + Demedull.					
	31	10	0	— 4	— 15	+ 260
	32	10	+ 12	+ 16	+ 46	+ 72
	33	10	+ 4	— 29	— 12	+ 25
	34	10	— 17	— 7	+ 12	0
31	Demedull.					
	16	10	+ 2	— 12	— 12	0
	19	10	0	+ 17	+ 6	+ 50
	21	10	— 9	— 18	— 8	+ 32
32	Demedull.					
	18	10	+ 1	+ 4	— 30	0
	20	10	— 17	— 19	— 10	0
89	Demedull.					
	11	10	— 9	— 28	— 12	0
	14	10	— 7	— 14	— 8	0

the number of granulocytes following injections of adrenaline are as capricious as those observed after ACTH and histamine.

Nor-adrenaline. Observations on four rabbits are given in Table 6. Nor-adrenaline appears less effective than adrenaline in causing lymphopenia, the response occurred less regularly, a temporary lymphocytosis was noted in three of twelve trials, the total pattern of response differs in five of the twelve tests from that seen with adrenaline. Contrary to adrenaline and other agents tested, nor-adrenaline did not induce granulopenia, granulocytosis or no change being the sole responses observed so far.

Discussion.

Histamine may reach the anterior pituitary gland by two routes: 1) from cerebral regions via the hypophyseal portal vessels, 2) by way of the systemic circulation.

The first possibility has been discussed in connection with the observation that cerebral regions related to the hypophysis (the hypothalamus, the median eminence of the tuber cinereum) and the anterior pituitary lobe itself contain histamine in high con-

centrations (HARRIS, JACOBSON and KAHLSON 1952). The physiological meaning of this finding is still obscure and has recently been discussed by SWINGLE, BRANNICK, BARRET, LE BRIE and PARLOW 1956. The part played by histamine as a chemotransmitter, if any, may possibly be revealed or repudiated by studies of the effect and constituents of blood from hypophyseal-portal vessels collected as described by PORTER and JONES 1956.

The present experiments are concerned with histamine carried by the general circulation. The effect of relatively small quantities of histamine only have been studied. Can the quantities used in the present investigation justly be referred to as physiological? It has been shown in the rabbit that the total amount of histamine liberated from various tissues and organs in severe antigen-antibody reactions is in the neighbourhood of 300 $\mu\text{g/kg}$ (SCHACHER 1953). It should further be considered that, apart from destruction of histamine by enzymes, a large proportion of histamine given by injection is captured by the tissues and kept there for some time (EMMELIN 1951). Under conditions of natural liberation of histamine, particularly when proceeding during a period much longer than the two minutes of injection, eventually an increasing proportion of liberated histamine will remain in the bloodstream, because the capacity of this capturing mechanism must be limited. Histamine injections into the carotid artery might give useful information on the minimum level of histamine effective in stimulating the anterior pituitary gland. This procedure is at present hardly practicable since the experiments must be done without anaesthesia and with quiescent animals, anaesthesia by itself causing lymphopenia.

The lymphopenic response to histamine is most likely due to increased secretion of the pituitary gland since hypophysectomy abolishes this response. Adrenaline, liberated by the injected histamine, is not essential to the response because removal of the adrenal medulla does not significantly alter the lymphopenic response to histamine. Further, the histamine doses used do not liberate effective quantities of adrenaline, since histamine did not induce lymphopenia after hypophysectomy, whereas injection of 10 $\mu\text{g/kg}$ adrenaline gave the response even after hypophysectomy.

Does histamine act directly on the pituitary gland or on cerebral regions controlling the activity of the anterior pituitary? It has been reported that the injection of 1,000 $\mu\text{g/kg}$ histamine in

hypophysectomized rats with adeno-hypophyseal tissue transplanted into the spleen or into the anterior chamber of the eye causes a fall in the adrenal ascorbic acid and in the number of blood eosinophils, respectively (CHENG, SAYERS, GOODMAN and SWINYARD 1949, FORTIER 1952). The physiological significance of these experiments is questioned on the ground that such enormous doses of histamine may inflict a nonspecific physico-chemical trauma to the cells of the pituitary tissue (GUILLEMIN 1955). In studies with Saffran's technique it was noted that histamine did not significantly increase the release of ACTH from isolated rat pituitaries in vitro (SHALLY and SAFFRAN 1956). This brings to the fore recent observations on the effect of histamine on cerebral structures. According to SAWYER (1955) injection of 0.25—0.5 milligram histamine (base) into the third ventricle in rabbits under nembutal anaesthesia is followed by electrical signs of "intrinsic olfactory activity" in the rhinencephalon. This author contends that histamine stimulates the adeno-hypophysis via rhinencephalic pathways and not by a direct action on the gland. TRENDELENBURG (1956) injected 5 to 100 μ g neutralized histamine dihydrochloride into the lateral ventricle of the cat's brain and recorded peripheral effects which, on closer analysis, suggested a direct action of histamine on the central ganglion cells of the sympathetic system. In this connection experiments by CROSSLAND and MITCHELL (1956) seem relevant. They found that injection of 0.04—0.08 μ g histamine into the internal carotid artery of the decerebrate rabbit during temporary occlusion of the vertebral arteries had an excitatory action on the cerebellum as recorded by changes in the electrical activity. Thus, histamine appears endowed with the inherent property, disclosed under special experimental conditions, of exciting cerebral structures.

Again, indicative of a direct action of histamine on the adeno-hypophysis are experiments by GRAY and MUNSON (1951). They showed that the fall in adrenal ascorbic acid which in the rat occurred after injection of 3—4 mg/kg histamine could be prevented by pre-treatment of the rat with cortical steroids which are known to depress the responsiveness of the adeno-hypophysis to various types of stimulation (SAYERS and SAYERS 1947). The site of action of histamine carried by the general circulation, whether directly on the gland or primarily on cerebral structures, could be more definitely established by investigating the lympho-

penic response to histamine in rabbits with the hypophyseal stalk transected. Experiments of this type are being planned.

Whatever the precise mechanism of the stimulation of the adenohypophysis by histamine, the present experiments suggest that histamine, carried by the general circulation in quantities which may occur under natural conditions, is a potent stimulus to increased pituitary-adrenal activity.

In completing this discussion the remarkable fact seems worth mentioning that in clinical medicine courses of histamine and of ACTH are employed concurrently or as alternatives in the treatment of various types of allergic disease. The mode of action of histamine in this field of therapy may well be to stimulate the pituitary gland to increased discharge of ACTH.

Summary.

1. The lymphopenic response to emotional stimulation and to injections of ACTH, histamine and adrenaline was investigated in unanaesthetized normal, hypophysectomized and demedullated rabbits.

2. Intravenous injection of histamine, 50 μ g per kilogram body weight, causes a significant lymphopenic response of the same type as the response following the injection of ACTH.

3. After hypophysectomy histamine fails to give distinct lymphopenic responses.

4. The histamine antagonist mepyramine did not abolish the lymphopenic response to histamine.

5. Adrenaline, liberated from the adrenal medulla, is not essential in the lymphopenic response to histamine or to emotional stimulation.

6. It is suggested that "physiological" quantities of histamine, when appearing in the general circulation, constitute an effective stimulus to increased secretion of ACTH from the adenohypophysis.

This investigation was supported by a grant from the Rockefeller Foundation. We should like to thank Dr. C. H. LI for giving us a supply of ACTH protein. We are most grateful to Dr. DORA JACOBSON who contributed essentially to this investigation by performing the hypophysectomy and the microscopic examination. The valuable technical assistance of Miss EDITH LEHMANN is gratefully acknowledged.

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Electrical Potential Gradients Through Frog Skin.

By

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Received 1 March 1957.

The potential difference across the isolated frog skin amounts to about 100 mV, the inner surface being positive. In previous microelectrode experiments (OTTOSON, SJÖSTRAND, STENSTRÖM and SVAETICHIN 1953) with frog skins in ordinary chloride-containing Ringer's it was found that only one potential jump accounted for the whole frog skin potential. Similarly LINDERHOLM (1954) assumed a single membrane to be the major diffusion barrier in frog skin. The skin potential is increased to a maximum of 150 mV when the chloride ion of the bathing solution is replaced by the non-penetrating sulphate ion. The effect on the frog skin potential of various sodium and potassium concentrations in the bathing solutions suggested that the frog skin potential in the absence of penetrating anions is the sum of a sodium diffusion potential at the outer border and a potassium diffusion potential at the inner border of the epithelial cell (KOEFOED-JOHNSEN and USSING 1956). This implies that the interior of the epithelial cell is at some intermediate potential and corresponding to the two cell borders one would expect two potential jumps on piercing the skin with a microelectrode. It seemed therefore of interest to investigate with microelectrodes the potential gradients of frog skins bathed in a solution in which the chloride was replaced by sulphate ions.

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Methods.

Preparations. The experiments were performed during the months May–September. Abdominal skin from the frog (*Rana temporaria*, *oxyrhina* or *esculenta*) was mounted across a perspex chamber with the outside surface facing up. The edges were clamped in a perspex ring separating the fluids outside and inside the skin from each other. The fluid bathing the inner surface of the frog skin was circulated with an airstream. The experiments were performed at room temperature (20°C).

Solutions. The outside and inside bathing solutions were sodium sulphate Ringer's, containing: 115 meq Na, 5 meq K, 2 meq Ca, 117 meq SO_4 and 5 meq phosphate, pH 7.9.

Electrodes and amplifiers. Ag-AgCl reference electrodes in chloride Ringer's solution were connected by Na_2SO_4 Ringer-agar bridges to the bathing solutions in the upper and lower chambers. The electrode to the upper chamber was grounded.

The potential difference between the two reference electrodes equalled the total skin potential.

Capillary microelectrodes filled with 3 M KCl (LING and GERARD 1949, NASTUK and HODGKIN 1950) were used for puncturing the skin. The total skin potential and the potential difference between the microelectrode and the grounded reference electrode were amplified with d. c. amplifiers with cathode follower inputs and visualized on a cathode-ray oscilloscope.

The microelectrodes were selected on the basis of impedance (4–23 megohms) and a small "tip potential". In agreement with R. H. ADRIAN (1956) we found a junction potential between the tip of the microelectrode and the test solution. The tip potential was always significantly more negative in sodium sulphate Ringer's solution than in 120 mM KCl solution. Microelectrodes with tip potential differences in the two solutions of less than 10 mV were selected.

Experimental procedure. The microelectrode was mounted in a micromanipulator and the insertion into the skin was made under forty times magnification. The skin was punctured perpendicularly from the outside, avoiding the openings of the skin glands, by advancing the microelectrode in steps of about 3–5 microns as measured by a gauge attached to the micromanipulator. The microelectrode was advanced in steps until the potential measured with the microelectrode approximated the total skin potential. The graph of the microelectrode potential change as a function of the distance from the surface was called a "potential profile". After the experiment electrode impedance and oscilloscope baseline were checked, and the experiment was rejected if the impedance was less than 3 megohms, or the baseline change was more than 5 mV. Skins with total potentials of less than 70 mV were rejected.

Histology. In fresh skins the total thickness was measured with a micrometer, and the epithelium thickness as the distance between

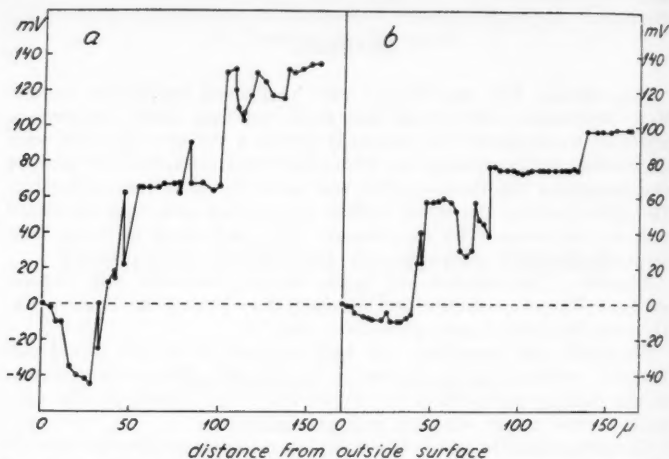


Fig. 1a and b. Frog skin potential profile in Na_2SO_4 -Ringer's. Abscissa: distance moved by microelectrode from first negative potential change in μ . Ordinate: microelectrode potential referred to the outside solution.

the superficial cornified cells and the chromatophors which could be seen through the epidermis at the junction between epidermis and corium.

Results.

Potential profile experiments. Fourteen skins bathed in sulphate Ringer's solution had total skin potentials of 73–145 mV (inside positive with reference to ground). Twenty-five punctures were made. In fifteen of these, profile patterns with two (Fig. 1a) or three (Fig. 1b) clear positive potential jumps were found, while three had two or more less well-defined jumps. In two only one positive potential jump was encountered and five showed gradual potential changes. In successive punctures in the same skin at different sites the profiles obtained were often quite different from each other, not only in the number of potential jumps but also in their magnitude.

In all instances but one the first potential encountered in the skin was a negativity (OTTOSON, SJÖSTRAND, STENSTRÖM and SVAETICHIN), which reached a maximum of 4 to 60 mV, and often showed some fluctuation during penetration. In the 15 profiles with well defined potential jumps the first positive potential jump appeared at a depth of 16–100 μ . As the micro-

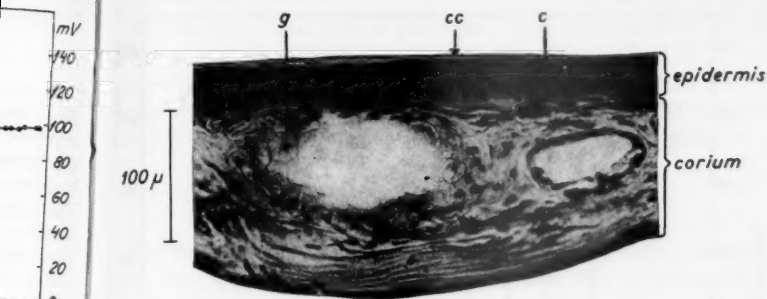


Fig. 2. Cross section of the skin of the frog (*Rana temporaria*, 4 per cent neutral formalin, hematoxylin-eosin, shrinkage ca. 35 per cent). c. Chromatophore cell. cc. Cornified cells. g. Skin gland.

electrode advanced the potential fluctuated around this value, and at 20–75 μ deeper in the skin the positive potential suddenly approximated the total skin potential in 11 punctures (Fig. 1a), the total distances moved by the microelectrode from the surface being 57–166 μ . In four instances there were three positive potential jumps, the last two jumps at 80–130 μ and 140–170 μ from the surface respectively (Fig. 1b). The distances between the potential barriers must be considered rough estimates since a certain amount of deformation of the tissue is probable.

In 21 of the 25 experiments the maximum positive potential recorded by the microelectrode was within 10 mV and in 16 it was within 5 mV of the total skin potential. The final deficit in the microelectrode potential could often only be obtained by a rather large movement of the microelectrode, and frequently did not come in a single jump but gradually.

Correlation between microelectrode position and skin structure.

In the experiments presented here there was no direct evidence of the position of the microelectrode in the frog skin. Indirect evidence was obtained from microscopic measurement of the different layers of fresh and fixed skins. The skin consists of two layers, epidermis and corium (Fig. 2). Epidermis is composed of 3–6 layers of epithelial cells: the flattened and cornified cells towards the surface give way to more irregular cells in the next layers; regular cylindrical cells (stratum germinativum) form the inner layer. Just beneath the epidermis is the corium, connective tissue containing chromatophores and numerous glands

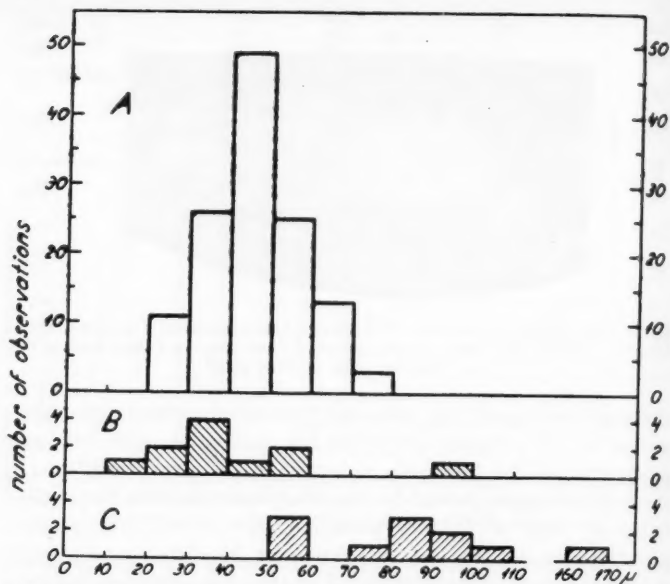


Fig. 3. Histograms of the thickness of frog skin epidermis (A) and sites of first (B) and second (C) potential jumps in 11 experiments with two potential jumps. Abscissa in A: thickness of epidermis in μ . Abscissa in B and C: distance moved by microelectrode from first negative potential change in μ .

which open at the epithelial surface. Measurements on fresh and fixed skins indicated that a shrinkage of up to 40 per cent occurred with the fixation employed. The positions of the potential jumps are therefore compared with the measurements on *fresh* skins, where only the distance from the surface of the epidermis to the middle of the chromatophors could be measured. The thickness of the epidermis (Fig. 3) was obtained by subtracting from this value the distance from the inner border of the epidermis to the middle of the chromatophors, measured in fixed skins. This latter distance was 10 μ , or 14 μ correcting for maximal shrinkage.

The positions of the first and second jump in the eleven cases with two positive jumps are indicated in Fig. 3. The site of the first positive jump occurs at a depth which corresponds to a position within the epidermis. The site of the second positive jump may correspond to the deep part of the epidermis or the superficial layer of the corium.

In the four cases with 3 potential jumps the positions of the first, second and third jump were 39—100 μ , 82—130 μ and 138—192 μ respectively. In these 4 cases the distances moved to the third jump were greater than the thickness of the epidermis possibly because of deformation or movement of the skin.

Discussion.

The first potential change encountered on penetrating the frog skin from the outside was a negative one; this is presumably a membrane potential of one or more of the cornified epithelial cells. The finding that the total skin potential is attained in two potential jumps is at variance with the findings of OTTOSON, SJÖSTRAND, STENSTRÖM and SVAETICHIN (1953). The different bathing solutions used in the two series of experiments cannot account for the discrepancy; a few experiments in this series with skins in chloride Ringer's showed the same potential profile as found in sulphate Ringer's. The two positive potential jumps indicate that the frog skin potential arises across two barriers at different depths in the skin. OTTOSON, SJÖSTRAND, STENSTRÖM and SVAETICHIN found the major jump accounting for the total skin potential in chloride Ringer's to be located across a sub-microscopic membrane demarcating epidermis from corium. This location may correspond to that of the second positive jump in the present experiments, while the first positive jump may be more superficial in the epidermis.

The finding of two potential jumps in the frog skin is in agreement with the theory of KOEFOED-JOHNSEN and USSING (1956), but the present experiments cannot decide whether these two jumps are situated at the outer and inner borders of the same epithelial cell as proposed by their theory. The distance moved between the first and second potential jump (20—70 μ) often exceeded the height of the cylindrical cell layer, but this might be due to deformation of the skin caused by a higher mechanical resistance at the junction between epidermis and corium. Were it assumed that the first potential jump is located at the outer cell border of the cylindrical cells, it would follow that the cell interior is positive referred to the outer extracellular space. If the second positive jump occurs as the electrode crosses the inner border of the same cylindrical cell a negative potential of the cell interior with reference to the inner extracellular space

would be indicated. A positive cell interior would be at variance with the usual finding of a negative intracellular potential in resting muscle and nerve cells. However the situation in the frog skin epithelial cells may differ from nerve and muscle since there is a transfer of sodium in one direction through the cells, which involves that the portion of the cell membrane facing the inner and outer skin surfaces have different properties with respect to sodium transport. Such a difference in the properties of different portions of the membrane of the same cell has been demonstrated for the active electroplate of *electrophorus* (KEYNES and MARTINS-FERREIRA 1953), where during discharge the potential across the membrane is positive inside at the nervous and negative inside at the non-nervous face.

Summary.

The electrical potential gradients through the frog skin bathed in sulphate Ringer's were studied with microelectrodes inserted through the skin from its outer surface.

The total skin potentials ranged between 73 and 145 mV, the inside positive with reference to ground. In most instances the total skin potential was reached in two, rarely three, distinct potential jumps. Comparison with measurements of the frog skin thickness indicated that the site of the first jump is in the epidermis while the second potential jump may correspond to the junction between epidermis and corium.

We wish to thank *Professor H. H. Ussing*, Laboratory of Zoophysiology, for suggesting this problem. The work was supported by grants from the *Michaelsen Foundation*, Copenhagen and the *Rockefeller Foundation*, New York.

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The Influence of Adrenaline, Nor-adrenaline, and Acetylcholine on the Electrocardiogram of the Isolated Perfused Guinea-pig Heart.

By

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Received 7 March, 1957.

The sympathomimetic catecholamines nor-adrenaline and adrenaline on the one hand, and acetylcholine on the other have been demonstrated in the mammalian heart muscle in varying concentrations. The chief sympathomimetic substance of the heart is nor-adrenaline (EULER 1946). Stimulation of the cardiac sympathetic nerves produces an increase of the total myocardial catecholamines which is due, specifically, to an accumulation of nor-adrenaline (OUTSCHOORN & VOGT, 1952, RAAB & GIGEE 1953). EULER (1952) postulates that chromaffin cells within the heart produce adrenaline, and concludes that adrenaline and nor-adrenaline may serve different functions.

The amount of acetylcholine is increased on stimulation of the cardiac vagus (ROTHSCHUH, 1954). On account of its cholinergic activity at vagal nerve terminals, acetylcholine exerts a specific action upon the heart muscle. In stimulating intracardiac synaptic sympathetic structures it also serves as a promotor of sympathetic activity (HOFFMANN, HOFFMANN, MIDDLETON & TALESNIK, 1945, HEYMANS and BENNATI 1949).

Although sympathetic activation, vagal stimulation and the effects of adrenaline, nor-adrenaline, and acetylcholine have been extensively studied in both mammalian and amphibian hearts, these subjects are still of considerable interest since the bioelectric potentials associated with the sympathetic and vagal influ-

ences are of great importance from a clinical point of view, especially in conditions where a separation between functional and structural heart diseases is necessary. The sympathetic and parasympathetic cardiac nerves have a widespread effect on the excitability, conductivity, and contractility of cardiac muscle and the fluctuations in the degree of tonic activity manifested by the sympathetic and parasympathetic cardiac nerves exert influence, among other things, upon the position and magnitude of the T wave deflection of the electrocardiogram as the terminal phase of membrane repolarization coincides with the T wave.

There is lack of agreement as regards the similarity or difference of the action of adrenaline and nor-adrenaline on cardiac rhythmicity, excitability, and conductivity. Also the action of acetylcholine on these processes is a matter of controversy. Because of the physiologic importance assigned to electrical manifestations in the heart we feel that this problem warrants further investigation.

This paper describes the electrocardiogram of the perfused, spontaneously-beating guinea-pig heart and compares its modification by adrenaline and nor-adrenaline, as well as by acetylcholine, which substances were given before and after administration of dibenamine and atropine, respectively. Isolated heart preparations were used in order to avoid the humoral influences and the nervous control as much as possible.

Material and Methods.

Male guinea-pigs weighing between 250 and 450 g were anesthetized with 18 to 30 mg nembital (Abbott) intraperitoneally. Tracheotomy was made and artificial respiration was begun when the animal's respiration became weaker. The thorax was opened as soon as possible and a glass cannula was inserted into the aorta. The perfusion fluid flowing through the cannula, contained, per liter: NaCl, 8.0 g; KCl, 0.2 g; $\text{CaCl}_2 + 6 \text{H}_2\text{O}$, 0.4 g; $\text{MgCl}_2 + 6 \text{H}_2\text{O}$, 0.2 g; NaHCO_3 , 0.2 g; $\text{NaH}_2\text{PO}_4 + 1 \text{H}_2\text{O}$, 0.06 g; and glucose, 1.0 g. The temperature was kept at 38°C . A mixture of 97.5 per cent oxygen and 2.5 per cent carbon dioxide was continuously bubbled through the solution, in which the pH ranged between 7.2—7.5. The heart was then dissected free, and open vessels were ligated. A perfusion pressure of about 80 cm of water was employed. With this method the heart could be taken out without arrhythmias or with only transient ones. A few cases with persisting arrhythmia were excluded.

After the heart had been connected to the perfusion chamber, one electrode was inserted into the fat tissue around the aorta. Two elec-

trodes were applied to the surface of the heart against the pericardium of the left and right ventricle, respectively. The electrodes were needle-formed and covered with a thin layer of cotton, soaked in perfusion fluid. In this way the heart was not hurt by the metal of the electrode. The potential differences between the left and right ventricles on the one hand and the aorta electrode on the other were registered in Leads I and II, respectively. A comparatively higher potential over the ventricles gave an upward deflection in the electrocardiogram. The potential difference between the left and right ventricles was registered in Lead III and a comparatively higher potential over the right ventricle gave an upward deflection in the electrocardiogram. The electrocardiograms were registered by means of an apparatus of type Elema Triplex.

For the experiments l-adrenaline (Rhône-Poulenc), l-nor-adrenaline (Rhône-Poulenc), and acetylcholine chloride (Roche) were used. The synthetic l-adrenaline and l-nor-adrenaline bases were dissolved in N/10 hydrochloric acid and diluted with distilled water. All dilutions were prepared immediately before the beginning of the experiments. The varying doses of the substances were administered by means of single injections and each dose was contained in a 1 cc volume. Through the injection this volume was further diluted with a volume of 3.5 cc of perfusion fluid in the perfusion chamber just above the heart. Injection pressure and injection time were kept as constant as possible. Without the test drug, 1 cc of the distilled water caused no electrocardiographic changes, or, in a few cases, slight ones.

During one experiment, 2 to 6 adrenaline injections were made on nine hearts, 2 to 13 nor-adrenaline injections on twelve hearts and 6 to 8 acetylcholine injections on five hearts. The adrenaline doses varied between 2.73×10^{-7} and 5.45×10^{-2} M, the nor-adrenaline doses between 2.95×10^{-7} and 5.91×10^{-3} M, and the acetylcholine doses between 5.50×10^{-8} and 5.50×10^{-3} M. The injections were given in increasing doses. The period between the injections was sufficiently long to allow the heart to return to the pre-injectional state, or nearly so.

Control material. After the hearts had been contracting for an equilibration period of 20 to 30 minutes, the heart rate averaged 172 beats/min. (range: 115–250 beats/min.). The P wave duration averaged 0.04 sec. (range: 0.02–0.04 sec.), the P wave amplitude in Lead II 0.6 mV (range: 0.3–1.2 mV), the P–R duration 0.08 sec. (range: 0.06–0.10 sec.), the QRS duration 0.02 sec. (range: 0.02–0.04 sec.) and the Q–T time 0.19 sec. (range: 0.13–0.24 sec.). Four T wave types appeared (Fig. 1).

The response to the test drug was always compared with the values obtained during the equilibration period just before the test.

In order to find out whether the changes in Q–T duration were secondary to the variations in heart rate or not, the Q–T duration at a certain heart rate before the injection was compared with the Q–T at the same heart rate various times after the injection. When this procedure could not be performed, the heart rates at the same Q–T duration were compared. The pre-injection Q–T durations

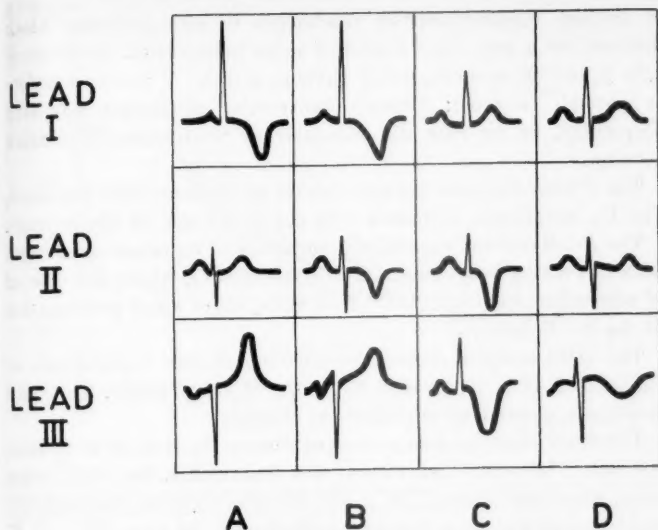


Fig. 1. Control electrocardiograms showing the configuration of the T waves in the experiments. Ten hearts belonged to group A, seven to group B, five to group C and four hearts to group D.

and corresponding heart rates were plotted, giving the Q—T/R—R relationship for the isolated, perfused guinea-pig heart. Both methods were used and gave consistent results.

Results.

Electrocardiographic changes after injection of adrenaline.

The threshold value for adrenaline to cause electrocardiographic changes, was 5.45×10^{-6} M in two hearts and 1.63×10^{-5} M in three hearts. In most cases the acceleratory effect was the first sign of the drug followed by changes in T waves and S—T segments.

The heart rate increased with 23 per cent at a dose of 1.63×10^{-5} M, and with 64 per cent at a dose of 1.36×10^{-5} M. The initial average heart rate before the appearance of the acceleratory effect was 176 beats/min.

Cardiac arrhythmias were registered in 10 of 32 experiments which showed electrocardiographic changes. The irregularities caused by lower doses (5.45×10^{-6} — 5.45×10^{-5} M) consisted

of ectopic supraventricular discharges or sino-auricular block followed by a very brief period of sinus bradycardia. At doses of 1.36×10^{-3} M or more, nodal rhythm and A—V block, complete or partial, occurred. Ectopic ventricular discharges appeared frequently. In no case did auricular or ventricular fibrillation develop.

The *P wave* duration became shorter as the heart rate increased. The P_{II} amplitude increased with 0.3 to 0.6 mV on the average.

The *P—R interval* was slightly shortened or remained unchanged with increasing heart rate. In six experiments, where the dose of of adrenaline was 1.36×10^{-3} M or more, there was a prolongation of the P—R interval.

The *QRS complex* showed lengthening in two experiments at a dose of 5.45×10^{-3} and 5.45×10^{-2} M, respectively. The QRS amplitude showed no unequivocal changes.

The *Q—T duration* was prolonged during the first 30 to 60 secs, thereafter, however, a shortening was found. (Fig. 2 a, b). In some cases, when large doses (1.36×10^{-3} M) were used, the prolongation could be followed by a shortening after only 30 secs. The Q—T values were usually restored within ten minutes.

Only slight deviations of the *S—T segment* were observed at lower doses. At higher doses, marked S—T displacement occurred which could be above or below the isoelectric level. After the injection of the drug, the previously negative and positive *T waves* became less negative or less positive, respectively (Fig. 3). At higher doses, the downward and upward T deflections diminished considerably and diphasic forms appeared. Occasionally, directional changes in the T occurred, i. e. a previously upright T became inverted or vice versa. The accompanying S—T deviations resembled an injury current with their monophasic complex. In most cases, T wave changes preceded the displacement of the S—T segment. Two hearts with positive T waves in tracings from the left and right ventricle, showed increased deflections after the drug; in one of these hearts, however, the changes were preceded by a transient decrease of the T wave deflection.

In most experiments, the high heart rate made it impossible to establish the existence of *U waves*. In some experiments, including those with total block, the heart rate was rather slow and no U waves could be seen.

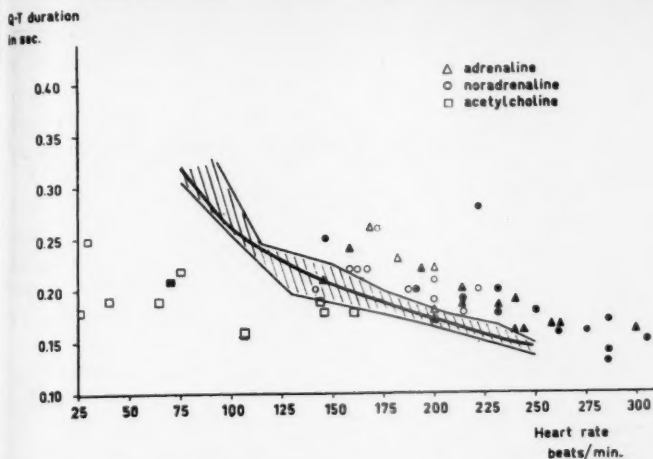


Fig. 2 a. Q — T duration in relationship to heart rate 30—60 secs after the injection of adrenaline, nor-adrenaline and acetylcholine. The values refer to the maximal prolongation for adrenaline and nor-adrenaline, and to the maximal shortening for acetylcholine.

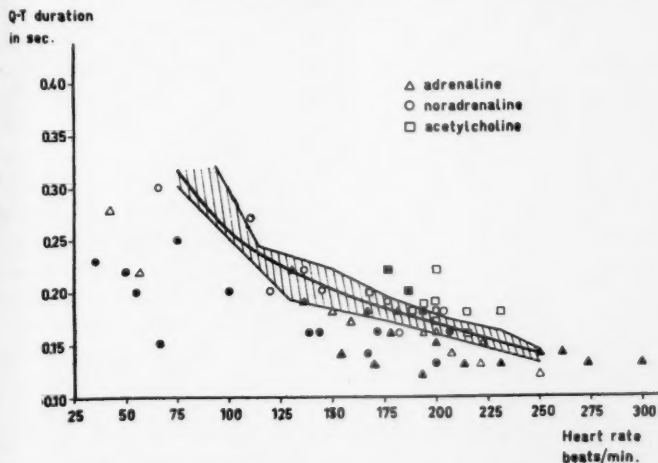


Fig. 2 b. Q — T duration in relationship to heart rate showing the maximal shortening after injection of adrenaline and nor-adrenaline, and the maximal prolongation after the injection of acetylcholine. These changes followed those illustrated in fig. 2 a.

All the pre-injection values of Q — T duration in relationship to heart rate fell within the shaded area. The mean values are represented by the heavy line within this area.

The open figures refer to a small dose while filled ones refer to a large dose.

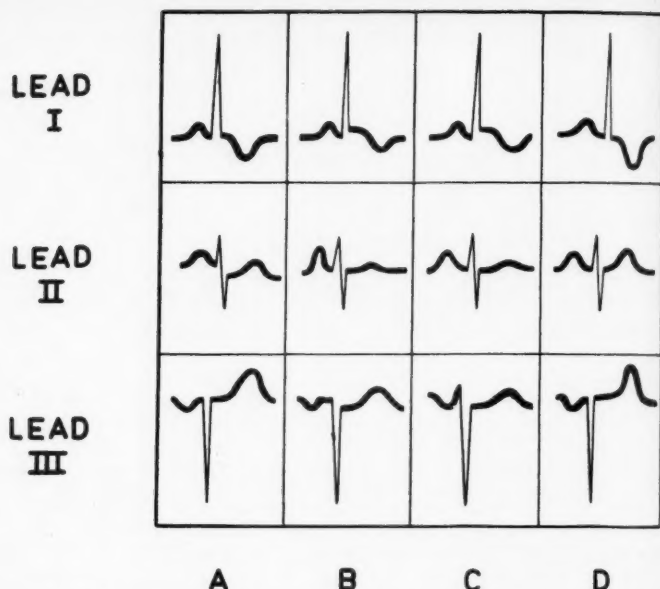



Fig. 3. Pre-injectional electrocardiogram (A). Prevailing contour changes of the S—T segments and T waves after injection of adrenaline (B), nor-adrenaline (C), and acetylcholine (D) after a small dose of the drug. For details, see the text.

Electrocardiographic changes after injection of nor-adrenaline.

The threshold doses for nor-adrenaline were 2.36×10^{-6} M in eight hearts and 5.91×10^{-6} M in two hearts. In most experiments, an increase of the heart rate was the first sign of nor-adrenaline action while electrocardiographic contour changes appeared later.

At a dose of 5.91×10^{-6} M the heart rate increased with 14 per cent and at 1.48×10^{-5} M with 102 per cent. The initial heart rate averaged 140 beats per min.

Cardiac arrhythmias could be registered in 17 experiments out of 44 which showed electrocardiographic changes. At lower doses, the evoked tachycardia was sometimes interrupted by a short period of irregular sinus rhythm or ectopic supraventricular discharges. Nodal escaped beats occurred in connection with these irregularities. At higher doses, the arrhythmias often consisted of heart block, complete or partial, and ectopic discharges



of nodal and ventricular origin. One heart exhibited a total block during the equilibration period before the test drug was given. In connection with repeated injections of nor-adrenaline, the ventricular rate increased and multifocal ventricular discharges appeared. When the drug caused a transient total block, the ventricular rate usually did not decrease. When a total block persisted after a few injections, further administration of nor-adrenaline caused an increase of the ventricular rate. In two cases ventricular tachycardia was noted. In one experiment, ventricular fibrillation occurred at a dose of 1.48×10^{-3} M. Forty minutes later it had disappeared spontaneously.

The *P wave* duration was reduced in connection with tachycardia. No significant changes in the P_{II} amplitude were registered.

The *P—R interval* remained unchanged or was slightly shortened with increasing heart rate. In a few experiments, however, a prolongation of the *P—R interval* appeared.

The *QRS complex* showed unchanged duration except in one experiment where a prolongation from 0.02 to 0.04 sec. occurred at a dose of 1.48×10^{-3} M. The *QRS amplitude* showed no unequivocal changes.

The *Q—T duration* showed a prolongation after 30 secs. After one minute a shortening was observed (Fig. 2 a, b). Normalization of the *Q—T duration* was usually noted within six minutes after the injection.

S—T deviations and *T wave* changes were similar to those caused by adrenaline. At lower doses the *S—T deviations* were absent. At higher doses the *S—T deviations* resembled an injury current with their monophasic complex. The minimal effect was a reduction in *T wave* amplitude: the previously negative or positive *T wave* became less negative or less positive, respectively (Fig. 3). In two hearts, the previously flattened, slightly diphasic *T wave* in Lead I became positive and the negative *T waves* in Leads II and III more negative after administration of nor-adrenaline.

No *U waves* were observed.

Electrocardiographic changes after injection of acetylcholine.

The threshold value for electrocardiographic changes was 5.5×10^{-7} M in all hearts except one where a dose of 5.5×10^{-6} M was necessary.

When acetylcholine was added in a dose just above the threshold value the *heart rate* decreased. Higher doses caused ventricular standstill and still higher doses were followed by auricular standstill, too. Ventricular standstill was registered in twelve experiments. In nine of these experiments the standstill was preceded by a brief period of decreased rate, in the others the standstill appeared suddenly.

Arrhythmias were frequent. Lower doses caused block of the first or second degree. With increasing doses total block and ventricular standstill occurred; still higher doses were followed by auricular fibrillation or auricular standstill. Auricular ectopic discharges occurred. Sometimes a brief paroxysm of auricular fibrillation could be registered as a series of rapid, fairly regular oscillations during the ventricular standstill. In experiments showing rapid auricular fibrillation this state was often accompanied by a regular ventricular rhythm. In these experiments the fibrillatory waves showed a frequency which could be as high as 1 800 to 3 000/min. As the effect of the drug wore off, the auricular fibrillatory waves either became coarser and accompanied by an irregular ventricular rhythm or gave way to sinus rhythm. In cases of auricular and ventricular standstill the auricles were the first to show activity. In a few experiments ectopic ventricular discharges could be registered during auricular standstill; ventricular discharges often initiated a more regular ventricular activity.

The *P wave* duration and amplitude decreased. In 10 experiments out of 33 showing ECG changes, the *P waves* became notched (saw-tooth appearance) and in 10 diphasic. Sometimes the saw-tooth appearance was the only sign of acetylcholine action.

The duration of the *QRS complex* remained unchanged and the amplitude showed no unequivocal changes.

The first change noted in the *Q—T duration* was a shortening, which in some cases after one minute was followed by a prolongation (Fig. 2 a, b). The pre-injection values were obtained after six minutes.

At lower doses no *S—T* deviations could be observed, at higher doses there were slight deviations preceded by *T wave* changes. In 20 experiments the previously positive or negative *T waves* became more positive and negative, respectively (Fig. 3). In five experiments, however, the *T waves* behaved the other way round.

In no experiments could a *U wave* be demonstrated.

Electrocardiographic changes caused by adrenaline and nor-adrenaline after the administration of dibenamine.

In one animal weighing 460 g, 10 mg of dibenamine (N-N-dibenzyl- β -chloroethylamine) were given intraperitoneally one hour before the experiment. The drug had no inhibiting action on any of the electrocardiographic changes caused by 5.9×10^{-5} M nor-adrenaline and 5.45×10^{-5} M adrenaline.

In other experiments on three hearts, dibenamine was dissolved in the perfusion fluid (20 mg dibenamine per liter perfusion fluid). This dibenamine concentration inhibited the action of low concentrations of adrenaline (1.63×10^{-5} M) and nor-adrenaline (5.91×10^{-5} M), sometimes also that of somewhat higher concentrations. When a large dose of adrenaline (1.36×10^{-3} M) and nor-adrenaline (1.48×10^{-3} M) was given, however, no inhibitory effect was found. In both experiments, ventricular tachycardia occurred and in the nor-adrenaline experiment also transient ventricular fibrillation.

Electrocardiographic changes caused by acetylcholine after the administration of atropine.

Two guinea-pigs weighing 350–400 g were treated with 1 mg of atropine one hour before the preparation of the heart. Acetylcholine at doses of 5.5×10^{-7} M and 5.5×10^{-6} M caused no electrocardiographic changes whatever. At a dose of 5.5×10^{-5} M a slight increase of the heart rate was noted in two experiments. S—T segments and T waves showed no changes.

Discussion.

Observations by Nathanson and Miller (1950) showed that nor-adrenaline had only a very slight direct chronotropic action on the human heart ventricles in complete block, while adrenaline produced a marked and sustained increase in the ventricular rate. In artificially driven hearts, BROOKS, HOFFMAN, SUCKLING and ORIAS (1955), however, could show that both nor-adrenaline and adrenaline increased the intrinsic ventricular rate in dogs and produced ectopic ventricular rhythms. In six experiments in the present investigation where a complete block developed and persisted after a few nor-adrenaline injections, repeated administrations of this substance caused a marked increase of the ventricular

rate and evoked multifocal ventricular discharges. When the drug caused a transient complete block during the tachycardia phase, the ventricular rate usually did not decrease.

The observations of RAAB (1956) on atropinized cats suggested that nor-adrenaline was less prone to evoke rhythmic disturbances than adrenaline. The present study, however, showed that nor-adrenaline is at least as potent as adrenaline in developing cardiac arrhythmias in isolated perfused guinea-pig hearts, which is in accordance with the investigations of GREINER and GARB (1950) on papillary muscles.

Adrenaline is said to prolongate the Q—T duration (LEPESCHKIN 1951). REMINGTON and AHLQUIST (1953) were unable to show that adrenaline or vagal stimulation had a specific effect upon the Q—T duration in experiments with anesthetized dogs. The present work showed that after administration of adrenaline and nor-adrenaline the Q—T duration was first prolonged and then after 30 to 60 secs. shortened. A similar result is described by GARB (1953). In this kind of experiments it is thus important to make continuous registrations. In addition, the usual formulas (those reported by BAZETT, ASHMAN, FRIDERICIA and SCHLAMOWITZ — for literature see LEPESCHKIN 1951) may give contrary results in determining the Q—T corrected for heart rate in animals of different kinds. It is important, therefore, either to make a special Q—T/R—R relation curve for the animal in question, or to compare the Q—T durations at the same heart rate, or the hearts rates at the same Q—T durations.

In experiments on atropinized cats, RAAB (1953) showed that adrenaline and nor-adrenaline caused a transient flattening or inversion of the T wave followed by an elevation. GARB (1953) on the other hand, was able to demonstrate a definite increase of the T deflection in isolated cat papillary muscle, followed by a depression or inversion of the T wave in some experiments. BROOKS, HOFFMAN, SUCKLING and ORIAS (1955) reported that adrenaline as well as sympathetic stimulation increased the voltage of the T wave in unipolar ventricular electrogram in dog. The results of the present study showed that in most experiments the minimal effect of adrenaline and nor-adrenaline was a reduction in the amplitude of the T wave: the previously positive or negative T wave became less positive or less negative, respectively. This reduction was at higher doses followed by a diphasic form and thereafter sometimes by directional changes, i. e., a previously upright T

wave became inverted or vice versa. In some hearts, however, the two substances caused an increased deflection of the T wave: the previously positive or negative deflection became more positive or more negative, respectively.

There is general agreement that acetylcholine causes a Q—T shortening (for literature, see LEPESCHKIN 1951). The present investigation showed that acetylcholine decreases the Q—T duration. In some experiments, however, a prolongation followed the initial decrease.

COHN and MACLEOD (1941) demonstrated a definite increase in the height of the T wave after administration of acetylcholine in the denervated dog; according to LEPESCHKIN (1951) this happened in animals when small doses were used, while larger doses might cause an inversion of the T wave. In frog heart acetylcholine caused an initial suppression or reversal of the T wave or made it disappear completely (BAKER and BAKER 1955).

In most experiments of the present investigation the T waves showed increased deflection after administration of acetylcholine, so that a previously positive or negative T wave became more positive or more negative, respectively. In some experiments, however, either in connection with repeated injections or after a prolonged ventricular standstill, the opposite was seen.

* * *

The mechanisms involved in cardiac function are very complex since they depend on factors which cannot be considered as independent variables.

Adrenaline appears to be involved in the regulation of the excitability of parasympathetic ganglia (MIDDLETON and TALESNIK 1949) and possibly involved in the production or activity of acetylcholine; on the other hand acetylcholine besides its cholinergic activity seems to stimulate the synaptic sympathetic structures in the myocardium (for literature, see RAAB 1953).

Lack of agreement of some reports concerning the action of adrenaline, nor-adrenaline, and acetylcholine may be due to 1) different experimental conditions, 2) differences between species, and 3) interpretation difficulties caused by the complex interaction between catecholamines and acetylcholine.

It cannot be assumed with certainty that experimental observations on animals would apply also to man. It would be of con-

siderable interest to study the bioelectric potentials associated with the sympathetic and vagal influences directly in the human cardiac muscle. Further studies on this subject are in progress.

Summary.

1. The effect of increasingly large doses of adrenaline, nor-adrenaline, and acetylcholine on the electrocardiogram of the isolated, perfused guinea-pig heart was studied before and after administration of dibenamine and atropine, respectively.

2. The threshold value for nor-adrenaline to cause electrocardiographic changes was lower than that for adrenaline. The chronotropic effect seemed to be the same for the two substances at low doses, while at higher doses nor-adrenaline seemed to be the more effective positive chronotropic agent. Cardiac arrhythmias were observed in about one-third of the experiments and nor-adrenaline appeared to be as potent as adrenaline in developing conduction disturbances and evoking ectopic discharges. Adrenaline increased the amplitude of the P wave, while nor-adrenaline had no effect on the amplitude. There was no difference between the two substances as to the changes in the P—R interval, Q—T duration, S—T segment, or on the T waves. At lower doses the P—R interval was shortened; a prolongation was seen at high doses. The Q—T duration was first prolonged, but 30 to 60 secs later a shortening appeared. The T waves showed reduction in amplitude, so that a previously positive or negative T wave became less positive or less negative, respectively. In some hearts the opposite results were seen. At high doses a previously positive T wave could become negative and vice versa.

3. After dibenaminization, slightly higher threshold values of adrenaline and nor-adrenaline were necessary in order to produce electrocardiographic changes. At higher doses of the two substances, dibenamine could not abolish or decrease the chronotropic effect or electrocardiographic contour changes.

4. At lower doses acetylcholine produced decreased auricular rate, with increasing doses heart block occurred, partial or complete. A propensity to evoke auricular fibrillation was evident. Ectopic auricular or ventricular discharges occurred. The P wave often became notched, its amplitude and duration decreased. In some experiments the Q—T duration was shortened and was followed by a lengthening. The T wave amplitude increased so that

a previously positive or negative T wave became more positive or more negative, respectively.

5. The inhibitory effect of acetylcholine was abolished by atropine. High doses of acetylcholine caused acceleration of the heart rate in atropinized hearts.

This work was supported by a grant from the Faculty of Medicine of the University of Lund.

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Enzymic Oxidation of Some Substances Related to Histamine.

By

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Received 7 March 1957.

It is known that there exist amines which are chemically related to histamine and which have pharmacological actions very similar to those of histamine (*i. a.* WALTER, HUNT and FOSBINDER 1941, SCHILD 1947, LEE and JONES 1949). The enzymic inactivation of these substances has received relatively little attention. ARUNLAKSHANA, MONGAR and SCHILD (1954) found that two substances examined by LEE and JONES, *i. e.*, 2- β -aminoethyl pyridine and 3- β -aminoethyl pyrazole, both substances with histamine-like action, were not inactivated by histaminase. This enabled ARUNLAKSHANA *et al.* to use these two substances to demonstrate the specific action of histaminase inhibitors in sensitizing isolated guinea-pig organs towards the action of histamine.

Observations on the effects of histaminase inhibitors on the response to histamine in cats and guinea-pigs have already been reported (LINDELL and WESTLING 1956, WESTLING 1956). In the course of these studies, it became desirable to investigate the action of histaminase and amine oxidase on some histamine-like substances. It was found that some of these substances were oxidized by enzyme preparations that contained amine oxidase, but that they were not oxidized by histaminase preparations.

Experimental.

Histaminase preparations. Partly purified histaminase was prepared from pig kidneys as described by ARVIDSSON, PERNOW and SWEDIN (1956). The procedure involved heating to 62° C for 10 minutes and precipitations with acetone and ammonium sulphate. The final precipitate was dissolved in distilled water and dialyzed for 48 hours against 2.5 % NaCl. This enzyme preparation did not oxidize sympatol, which was used as a typical substrate of amine oxidase.

An extract of cat kidney, which oxidized histamine but not sympatol could be obtained by centrifuging a crude tissue extract in 0.067 M sodium phosphate buffer (pH 7.4; 2 ml of buffer for 1 g of tissue) at 25 000 × g for 2 hours. The supernatant was pipetted off; it is referred to as cat kidney histaminase preparation in the text.

Amine oxidase preparations. Preparations with amine oxidase activity were obtained from guinea-pig and rabbit liver and from cat kidney. The fresh tissue was ground thoroughly in a mortar and 0.067 M sodium phosphate buffer (pH 7.4) added (2 ml of buffer for 1 g of tissue). The preparations were dialyzed for 18–24 hours against running tap water and then stored at -20° C.

Measurement of oxygen consumption. Oxygen consumption was measured manometrically by the Warburg method. The enzyme preparation (0.3 ml of the pig kidney preparations; 1.0–2.4 ml of the others) and a suitable amount of 0.067 M sodium phosphate buffer (pH 7.4) were put in the main compartment of the Warburg vessels. The central tube contained 0.3 ml of 1 M KOH. The solution of substrate or the same volume of distilled water (for "enzyme blank") were put in a side bulb. Inhibitors were usually tipped in from a separate side bulb at the same time as the substrate was added. In some experiments, mentioned in the text, the inhibitor was present in the enzyme solution during the whole period of temperature equilibration. Readings were taken every 5 minutes. None of the substances used were oxidized in the absence of enzyme preparations. The oxygen consumption caused by the enzyme preparations in the absence of substrate ("enzyme blank") was subtracted. The oxygen consumption and the percentage inhibition were, if possible, calculated from readings taken during the first 15 minutes of the reaction. During this time the oxygen uptake was approximately linear.

The initial concentrations of substrate, after tipping, were 0.01 M in the experiments with amine oxidase, in those with histaminase either 0.01 M or 0.002 M. Substrates and inhibitors were dissolved in distilled water and the solutions neutralized with NaOH (to give green colour with bromothymol blue).

The influence of cyanide ions on oxygen consumption was studied in some experiments. A solution of $\text{Ca}(\text{CN})_2 + \text{Ca}(\text{OH})_2$ was prepared and analyzed for cyanide content as described by ROBBIE (1948). Amounts of $\text{Ca}(\text{CN})_2$, calculated by ROBBIE to be in equilibrium with 10^{-3} M of cyanide in the enzyme solution, were put in

the central tube of the flasks. Neutralized KCN was added to the enzyme preparation to make a final concentration of 10^{-3} M.

The temperature of the water bath was 37.5° C. The gas phase in all experiments was oxygen. Glass-redistilled water was used.

Measurement of "ammonia". In some experiments with guinea-pig liver preparations the amount of volatile base formed during the manometric experiment was measured. The method for determining the amount of volatile base was essentially the same as that described by COTZIAS and DOLE (1951). In a manometric experiment, when a suitable amount of oxygen had been taken up, the manometer with substrate and enzyme and a corresponding one containing "enzyme blank" were quickly taken out of the bath and opened; 0.5 ml of the reaction fluid were rapidly transferred to the outer compartment of a Conway unit. The inner chamber of the Conway unit contained an indicator solution (boric acid with methyl red and bromocresol green). The outer compartment contained 1 ml saturated potassium metaborate within silicone walls. The units were sealed and reaction fluid and metaborate mixed. The units were left for 2 hours at room temperature for equilibration. The indicator solution was then pipetted off quantitatively and titrated back to the original colour with 0.01 N HCl. A microsyringe (Burroughs Wellcome) was used for the titrations. Amounts of volatile base found in the "enzyme blank" and in the substrate solutions were subtracted.

Substances.* L-p-sympatol hydrochloride, histamine acid phosphate, cadaverine dihydrochloride, aminoguanidine sulphate, semicarbazide dihydrochloride and 1-isonicotinyl-2-isopropyl hydrazine (iproniazid, Marsilid Base "Roche") were used.

The 7 histamine-like substances investigated were all used as the dihydrochlorides. The pharmacological actions of 6 of these compounds have been studied by LEE and JONES (1949); the latin figures are the numbers given by these authors to the different compounds. The substances were: 1-methyl-4- β -aminoethyl imidazole (referred to as methylhistamine, III), 4- β -aminoethyl thiazole (V), 2- β -aminoethyl pyridine (VIII), 2- β -aminoethyl pyrimidine (IX), 2- β -aminoethyl imidazole (XIII) and 3- β -aminoethyl pyrazole (XXIV). The seventh substance studied was 3- β -aminoethyl-1, 2, 4-triazole, described by AINSWORTH and JONES (1953). The structural formulae of the substances examined and their relative histamine-like activities are given in fig. 1.

Results.

1. *Histaminase preparations.* All the amines were incubated with the partially purified histaminase preparation from pig kidney. The results are shown in fig. 1, which gives the

* We are most grateful to dr. Reuben G. Jones of the Eli Lilly Research Laboratories, Indianapolis, Indiana, U.S.A., who gave us the histamine-like substances. Hoffmann-La Roche AG, Basel, Switzerland, kindly supplied us with iproniazid.

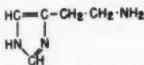
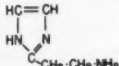
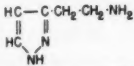
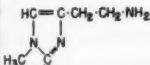
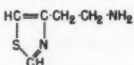
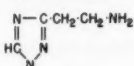
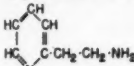
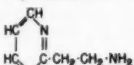
SUBSTANCE	NO.	HISTAMINE-LIKE ACTIVITY	OXIDATION BY "AMINE OXIDASE"	BY "HISTAMINASE"
HISTAMINE 	—	100	0	100
2-β-AMINOETHYL IMIDAZOLE 	XIII	0	0	10
3-β-AMINOETHYL PYRAZOLE 	XXIV	0	11	9
1-METHYL-4-β-AMINOETHYL IMIDAZOLE 	III	0.6	18	150
4-β-AMINOETHYL THIAZOLE 	V	2	32	0
3-β-AMINOETHYL-1,2,4-TRIAZOLE 	—	17	0	0
2-β-AMINOETHYL PYRIDINE 	VIII	9	32	0
2-β-AMINOETHYL PYRIMIDINE 	IX	5	25	0

Fig. 1. Structural formulae of the histamine-like substances examined and their respective numbers after Lee and Jones (1949). The relative histamine-like activity on the guinea-pig's isolated ileum (after Lee and Jones, 1949, and Ainsworth and Jones, 1953) is given in the first column. The figures in the second and third columns give the oxygen uptake with amine oxidase preparations (guinea-pig liver) and histaminase preparations (pig kidney) as per cent of that of an equimolar amount of sympatol and histamine, respectively.

oxygen consumption noted with the various substances in per cent of that with an equimolar solution of histamine. The oxygen consumption with 2×10^{-3} M histamine was about 25 mm³ during the first 15 minutes. An oxygen uptake was found with three of the substances tested, *i. e.*, with compounds III, XIII and XXIV. The action on compound III will be described later in this section. The oxygen uptake caused by compounds XIII and XXIV was about 10 % of that of histamine. Each one of the amines tested depressed the oxidation of histamine, when added simultaneously to the enzyme preparation. Compound XIII was most effective in causing a depression; it reduced the oxidation of histamine (initial concentration 2×10^{-3} M) by about 50 % when present in a concentration of 8×10^{-5} M. Compound XIII also depressed strongly the oxidation of cadaverine by the pig kidney histaminase preparation. The percentage reduction of the oxidation of histamine, caused by the other substances was smaller; in a concentration of 2×10^{-3} M it varied between 17 and 28. Compound XIII was thus found to be a strong inhibitor of histamine oxidation. Although weaker than aminoguanidine (see table 2), it was about as effective as semicarbazide, which gave 50 % inhibition at a concentration of about 10^{-4} M and identical conditions.

Two of the substances that were not oxidized by the pig kidney histaminase, 3- β -aminoethyl-1, 2, 4-triazole and 2- β -aminoethyl pyridine (VIII), were also studied with the histaminase preparation from cat kidney. This enzyme preparation oxidized both cadaverine and histamine but addition of the triazole compound or compound VIII gave no measurable oxygen uptake.

Methylhistamine (compound III) was found to be oxidized by the partially purified pig histaminase preparation at a higher rate than histamine (table 1). The oxygen uptake by methylhistamine was about half of that seen with an equimolar amount of cadaverine. When methylhistamine and cadaverine were added simultaneously to the enzyme preparation the oxygen uptake was competitive, not additive (fig. 2). The same results were obtained with methylhistamine versus histamine. When methylhistamine and histamine were added simultaneously to the enzyme preparation in concentrations of 10^{-2} M the oxygen uptake was also intermediate between those with the two amines, when studied separately. The oxida-

Table 1.

Comparison of the oxidation of cadaverine, histamine and methylhistamine by a histaminase preparation (pig kidney). The figures given are mm³ of oxygen consumed during the first 15 min.

Substrate	initial substrate conc. 10^{-2} M	initial substrate conc. $2 \cdot 10^{-3}$ M
cadaverine	63	49
histamine	7	16
1-methyl-4- β -aminoethyl imidazole	30	26

Table 2.

Inhibition by aminoguanidine of oxidation of cadaverine, histamine and methylhistamine by a histaminase preparation (pig kidney). Initial substrate concentrations $2 \cdot 10^{-3}$ M. The aminoguanidine was added simultaneously with the substrate.

Substrate	% inhibition by aminoguanidine			
	10^{-4} - M	10^{-5} - M	10^{-6} - M	10^{-7} - M
cadaverine	100	98	76	11
histamine	82	39	18	10
1-methyl-4- β -aminoethyl imidazole	97	45	13	3

tion of the methylated derivative was, in contrast to that of histamine, not diminished at a concentration of 10^{-2} M (table 1). The oxidation of methylhistamine was inhibited by aminoguanidine in the same degree as was the oxidation of histamine (table 2).

Methylhistamine was also oxidized by a chromatographically purified histaminase preparation from pig kidney, the relations between the oxidations of cadaverine, methylhistamine and histamine being essentially the same as found with the less pure preparation*.

The action on methylhistamine of the preparation of histaminase from cat kidney was studied in one experiment. This preparation did not oxidize sympatol. Methylhistamine and

* The chromatographically purified histaminase preparation from pig kidney was kindly given to us by dr. B. Swedin. It had been prepared as described by Arvidsson, Pernow and Swedin (1956).

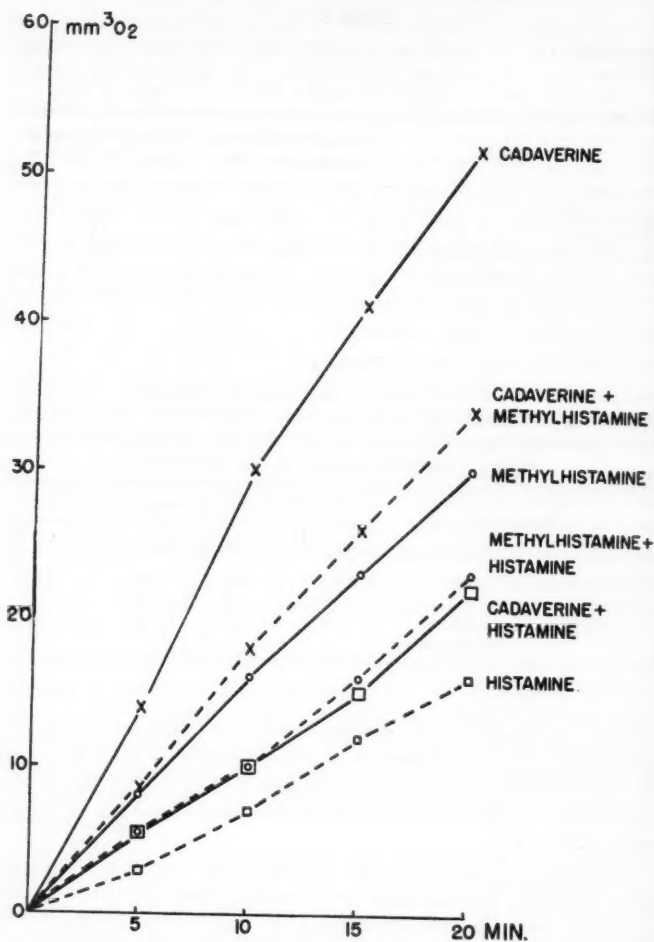


Fig. 2. Oxidation of cadaverine, histamine and 1-methyl-4- β -aminoethyl imidazole (methylhistamine) by a pig kidney histaminase preparation; initial substrate concentrations 2×10^{-3} M.

histamine were oxidized at about the same rate; the oxygen uptake with cadaverine as substrate was 2.5 times that with histamine.

² **Amine oxidase preparations.** The results of the experiments with preparations of amine oxidase from guinea-pig or rabbit liver and from cat kidney are given in table 3. Histamine was not oxidized by the liver preparations with the incubation times used. The cat kidney extracts, however, oxidized both histamine and cadaverine, presumably because they contained histaminase (LINDAHL, LINDELL, WESTLING and WHITE 1957) in addition to amine oxidase. To exclude the action of histaminase, the experiments with cat kidney preparations were carried out in the presence of aminoguanidine (10^{-4} M), added to the enzyme 20 minutes before adding substrates. The addition of 10^{-4} M aminoguanidine abolished the oxygen uptake with both cadaverine and histamine.

The rate of oxygen uptake by preparations of amine oxidase in the presence of a histamine-like substance was always compared with the rate in the presence of an equimolar amount of sympatol. It may be seen from table 3 that all substances except 3- β -aminoethyl triazole and compound XIII caused a small oxygen consumption. The rate of oxidation of the histamine-like substances, as compared with sympatol, varied slightly in different enzyme preparations from the same tissue. This variation was more marked for methylhistamine. In different preparations of guinea-pig liver the relative rate of oxidation of methylhistamine varied from 10 to 30 per cent of the rate of oxidation of sympatol. When a histamine-like substance was added together with sympatol the rate of oxygen uptake was intermediate between the rates for histamine-like substance and sympatol, when studied separately. The substances with a sixmembered ring (compounds VIII and IX) were more effective than the others in depressing sympatol oxidation by guinea-pig liver preparations.

Some species differences in the relative rates of oxidation of the different substances were observed. Thus compound V was oxidized at twice the rate of sympatol by rabbit liver, whereas with guinea-pig liver the rate of oxidation was one-third of that of sympatol; compound VIII was oxidized by cat kidney preparations at about the same rate as sympatol, but by the rabbit and guinea-pig enzyme at about one-third of the rate with sympatol.

The oxygen uptakes observed in these experiments were tentatively ascribed to the action of the amine oxidase present in the crude tissue preparations used. In order to test this

Table 3.

Oxidation of histamine-like substances by amine oxidase preparations from guinea-pig and rabbit liver and from cat kidney. The values refer to the oxygen uptake expressed as per cent of that seen with sympatol (25—80 mm³ O₂) during the first 15 min. The figure within parentheses represents the per cent change in oxygen uptake from that of sympatol, when the histamine-like substance and sympatol were incubated simultaneously. Initial concentration of all substances was 10⁻² M. The figures are the mean values of results of two or more experiments.

Substances	No.	Enzyme preparation		
		guinea-pig liver	rabbit liver	cat kidney
2-β-aminoethyl imidazole	XIII	0 (-19)	-	-
3-β-aminoethyl pyrazole	XXIV	11 (-9)	-	-
1-methyl-4-β-aminoethyl imidazole	III	18 (-36)	36 (-38)	34 (-51)
4-β-aminoethyl thiazole	V	32 (-20)	200 (+175)	-
3-β-aminoethyl triazole	—	0 (-25)	-	0 (± 0)
2-β-aminoethyl pyridine	VIII	32 (-52)	33 (-65)	110 (+5)
2-β-aminoethyl pyrimidine	IX	25 (-68)	20 (-80)	-

hypothesis it was attempted to find out whether the reaction was accompanied by a formation of ammonia. The amount of volatile base formed during the reaction was measured using the method of COTZIAS and DOLE (1951). The oxidation of sympatol and of compounds V, VIII and IX and methylhistamine by guinea-pig liver preparations was accompanied by the formation of a volatile base. The ratio between micromoles of volatile base formed and micromoles of oxygen consumed varied between 1.1 and 1.9. When sympatol and a histamine-like substance were incubated together the amount of volatile base formed was intermediate (fig. 3).

The oxidation of the substances tested was not affected by 10⁻⁴ M aminoguanidine added 20 min. before the substrates. In the presence of 10⁻² M semicarbazide the oxygen uptake of guinea-pig liver preparations with sympatol as substrate was reduced by 20—40 per cent; similar percentage inhibitions were seen with the histamine-like compounds. However, in an experiment with rabbit liver, 10⁻² M semicarbazide caused an inhibition of the oxidation of compound V of as much as 56 per cent, while the percentage inhibition with sympatol as substrate was 22 per cent.

MICROMOLES

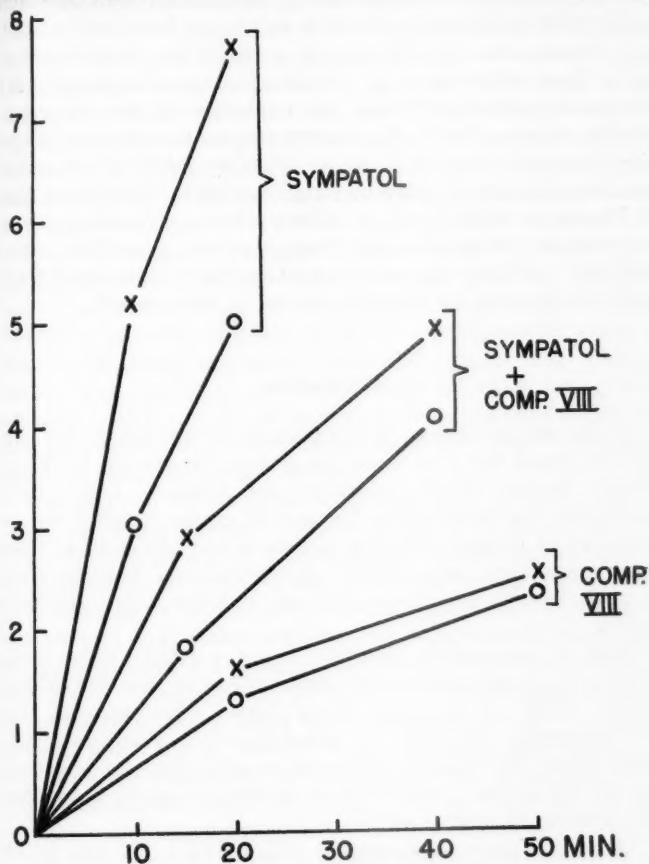


Fig. 3. Oxygen consumption and formation of volatile base by an amine oxidase preparation from guinea-pig liver. Substrates sympatol and 2- β -aminoethyl pyridine (comp. VIII) in 10^{-2} M concentration. Blank values have been subtracted.

x — x micromoles of volatile base formed.
o — o micromoles of O₂ consumed.

Some additional experiments with the effect of inhibitors on the oxidation of sympatol, 2- β -aminoethyl pyridine and methylhistamine were made with guinea-pig liver and cat kidney preparations. 10^{-3} M cyanide reduced the oxygen uptake with these substances by 10–30 %. Iproniazid (10^{-3} M) almost completely inhibited the oxidation of the three substances, when added to the enzyme preparation 30 min. before the substrate. Iproniazid is an effective inhibitor of amine oxidase (ZELLER, BARSKY and BERMAN 1955). Methylene blue is known to inhibit amine oxidase (PHILPOT 1937). In one experiment with a guinea-pig liver preparation, methylene blue (10^{-4} M) inhibited the oxidation of sympatol, compound VIII and methylhistamine by 83, 71 and 50 %, respectively.

Discussion.

As far as the action of histaminase is concerned, the substances tested fall into three categories. Compounds XIII and XXIV, derivatives of imidazole and pyrazole, respectively, were very slowly oxidized. The second group includes the derivatives of thiazole, triazole, pyridine and pyrimidine; these substances were not oxidized by histaminase. Thirdly, there is methylhistamine; it was oxidized by histaminase relatively rapidly.

That 2- β -aminoethyl pyridine is not a substrate of histaminase, is in agreement with observations by ARUNLAKSHANA *et al.* (1954). WERLE and PALM (1953) have reported that 2- β -aminoethyl pyridine is a substrate of a diamine oxidase from plants, but it must be borne in mind that the substrate specificity of the plant enzyme differs in many ways from the enzyme of animal origin.

It was found that compounds III, V, VIII, IX and XXIV were oxidized when they were incubated with dialyzed preparations of liver (guinea-pig and rabbit) and kidney (cat). This oxidation cannot be explained on the basis of histaminase activity, as the liver preparations contained only little histaminase. Moreover, the oxygen uptake was not affected by aminoguanidine, a substance known to be a strong inhibitor of histaminase (SCHULER 1952); this inhibition of the enzymic oxidation of both histamine and cadaverine by pig kidney enzyme was readily confirmed in the present experiments. The

enzyme preparations, which acted upon the compounds tested, all contained the enzyme amine oxidase; they oxidized sympatol, a characteristic substrate of amine oxidase. That amine oxidase was the catalyst responsible for the oxidation of these compounds, is also supported by the observation that in the presence of both sympatol and one of these compounds the rate of oxygen consumption was competitive and not additive. Furthermore, it was found that the oxidation of the histamine-like substances by guinea-pig liver was accompanied by a concomitant formation of a volatile base, probably ammonia.

The oxidation of sympatol, 2- β -aminoethyl pyridine and methylhistamine was inhibited by iproniazid, a substance known to inhibit amine oxidase (ZELLER *et al.* 1955); it must be borne in mind, however, that this inhibitor also blocks a number of other metabolic pathways (FOOTS and BRODIE 1956). The results of the experiments in which the effects of semicarbazide, cyanide and methylene blue were studied, are also in accord with the hypothesis that these substances were oxidized by amine oxidase.

The enzymic oxidation of compound III, methylhistamine, is of particular interest because it has been shown that in many species histamine is methylated to form this compound (SCHAYER and KARJALA 1956). The methylated histamine is then oxidized to form 1-methyl-imidazole-4-acetic acid, which is excreted in the urine. The present experiments show that methylhistamine is a good substrate of histaminase from cat and pig *in vitro*, and that its oxidation by these preparations of histaminase is inhibited by aminoguanidine. In the living animal, however, the formation of 1-methyl-imidazole-4-acetic acid was not diminished by aminoguanidine (SCHAYER, KENNEDY and SMILEY 1953). Amine oxidase may take part in the oxidation of methylhistamine *in vivo* (SCHAYER and KARJALA 1956), since its oxidation is inhibited by iproniazid and by 1-isonicotinyl-2-isobutyl hydrazine, both substances known to inhibit amine oxidase. The oxidation of methylhistamine, however, is not affected by the p-tolyl ether of choline (SCHAYER and KARJALA 1956), an inhibitor of amine oxidase in intact animals (SCHAYER, WU, SMILEY and KOBAYASHI 1954). There is, however, some disagreement how active this substance is as an inhibitor of amine oxidase *in vivo* (CORNE and GRAHAM 1957). As has been discussed above, it seems likely that methylhistamine as well as the other histamine-like substances was

oxidized by amine oxidase *in vitro*. However, the relative rates of oxidation of sympatol and methylhistamine varied somewhat and this might indicate that the enzyme responsible for the oxidation of methylhistamine was, after all, not identical with amine oxidase.

It is apparent from fig. 1 that there was no relation between the pharmacological activity of these substances and their oxidation by the enzyme preparations tested.

It has been pointed out that the degree of ionization of the two basic groups may be one of the factors that determine the affinity of diamines to histaminase and to amine oxidase (BLASCHKO, CHATTERJEE and HIMMS 1955). The pK_a values for some of the amines tested in this study were determined by Dr. Reuben G. Jones, who has kindly given us his data. Judging from these data, compounds V, VIII and IX must have been present mainly as mono-cations in our experiments. In agreement with this, they were oxidized by amine oxidase. The pK_a values of methylhistamine and those of compound XIII did not differ much from those of histamine.

One of the purposes of the present investigation was to find a substance which was not inactivated by histaminase or amine oxidase and which had histamine-like actions. The substance 3- β -aminoethyl-1, 2, 4-triazole seems to meet these requirements. On isolated tissues it is one of the more potent histamine analogues (AINSWORTH and JONES 1953).

2- β -aminoethyl pyridine seems to be unaffected by histaminase preparations from pig or cat and its histamine-like actions have not been potentiated by histaminase inhibitors in different types of experiments (ARUNLAKSHANA *et al.* 1954, LINDELL and WESTLING 1956, WESTLING 1956 and 1957 a). It has been observed (WESTLING 1957 b) that the histamine-like actions of 2- β -aminoethyl pyridine on intact guinea-pigs were potentiated by iproniazid. The present experiments provide a possible explanation of this observation, since they showed that 2- β -aminoethyl pyridine was oxidized by guinea-pig liver preparations. The oxidation was inhibited by iproniazid and the enzyme responsible was most likely amine oxidase.

Summary.

1. The action of histaminase and amine oxidase preparations on seven histamine-like substances was examined. The enzyme preparations were obtained from pig and cat kidney and from guinea-pig and rabbit liver.

2. Some of the substances studied were found to be oxidized by amine oxidase preparations but not by histaminase.

3. 1-methyl-4- β -aminoethyl imidazole was found to be a good substrate of histaminase. It was also oxidized by amine oxidase preparations.

We are much indebted to Dr. H. BLASCHKO, Oxford, for constant encouragement and valuable advice. Our thanks are also due to mag. scient. KAREN NILSSON, Lund, for skilful help.

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